"SYNTHESIS AND STRUCTURAL INVESTIGATION OF COORDINATION COMPOUNDS OF PLATINUM (II) WITH SUBSTITUTED THIAZOLES AND TRIAZOLES"

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CERTIFICATE OF SUPERVISOR

This is to certify that **Pratibha Shukla,** Research scholar in Chemistry, Atarra Post Graduate College Atarra (Banda) has worked under my supervision for the degree of **Doctor of Philosophy in Chemistry on the topic "Synthesis and Structural Investigation of Coordination Compounds of Platinum (II) with Substituted Thiazoles and Triazoles". She put her attendance more than 200** days in the department. The accompanying thesis submitted by her embodies the work of the candidate herself and it is an original work.

Dated : Sept. 25, 2006.

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Date: Sept. 25, 2006

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CONTENTS

PREFACE

The inception of coordination compounds of platinum starts with the synthesis, isolation and their antitumor properties was very limited until the discovery of certain platinum coordination compounds by Rosenberg and Van Camp in 1969. Extensive studies appears to have been made on synthesis and stereochemistry of coordination compounds of Molybdenum, Ruthenium, Rhodinium, Tungston, Osmium and Palladium etc. But, however, a little work seems to have been reported on the synthesis and stereochemistry aspects of coordination compounds of platinum in bivalent oxidation state with substituted azoles. It is, therefore, thought worthwhile to synthesize and characterized some coordination compounds of platinum with different biologically active organic compounds in II oxidation state, and results of this investigation are incorporated in this thesis.

The thesis entitled **"Synthesis and Structural Investigation of Coordination Compounds of Platinum [II] With Substituted Thiazoles and Triazoles"** consists of five chapters and the contents of each are as follows:

The Chapter I begins with the general introduction in which the existing literature of the coordination compounds of transition metal has been briefly surveyed. This chapter also consist the chemistry of coordination compounds of platinum with various biologically active ligands that have been reviewed in the past. Some recent literature concerning the basic core for the synthesis and structural studies of

platinum [II] compound reveals same report. As on outcome of these the scope of the present work has also been stretched.

The Chapter II describes the synthesis and physio-chemical studies of some coordination compounds of platinum with 2-aminothiazole, benzothiazole and 2-methyl-benzothiazole. The properties of the synthesized compounds have been studied in detail and the probable structure discussed on the basis of various physico-chemical studies.

The Chapter III deals with the synthesis, properties and structural investigations of coordination compounds of platinum [II] with 2-amino-6-methyl benzothiazole and 2-amino-4-methyl benzothiazole.

The Chapter IV describes the synthesis and structural investigations of coordination compounds of platinum (II) with 1,2,4-triazole, 3-amino-1, 2,4-triazole, 5-methyl benzotriazole, 5-nitro benzotriazole.

The last Chapter that is Chapter V illustrates the biochemical studies of synthesized coordination compounds. This chapter also describes and summarizes the biochemical and antitumor studies of metal complexes. The references given in this thesis are covered upto date.

ABBREVIATIONS

am	amine
bipy	2, 2` -bipyridine
dien	diethylenetriamine
L	ligand
DMSO	dimethylsulfoxide
en	ethylenediamine
EXAFS	extended x-ray absorption five structure
CNDO	complete neglect of differential overlap
IR	infrared
NMR	nuclear magnetic resonance
HET	2-hydroxyethanethiolate
MeIm	N-methylimidazole
o-phen	o-phenanthroline
PUB	platinum uracil blue
py	pyridine
tbp	trigonal bipyramidal
terpy	2, 2`, 2`` - terpyridine
DMG	dimethylglyoxime
cisplatin	cis-$[PtC_2(NH_3)_2]$
Me mal	2-methylmalonate
Et mal	2-ethylmalonate
DMF	dimethylformamide
2-ATZ	2-aminothiazole
BZT	benzothiazole
1,2,4-TAZ	1,2,4-triazole
BZT_R	benzotriazole
Mal	malonate

OH mal	2-hydroxymalonate 1,1-CBDCA 1, 1-cyclobutanedi-carboxylate
1,2-DAC	1,2-diaminocyclohexane
MTD	minimum toxic dose
MED	minimum effective dose
BUN	blood urea nitrogen
WBC	white blood cell amount
% T/C	% treated/controls
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
MDST	median survival time
MST	mean survival time
MTW	mean tumor weight
ESCA	electron spectroscopy for chemical analysis
TI	therapeutic index
$(LD_{50}/ID_{90})LD_{50}$	dose that kills 50% of a group of animals
ID_{90}	dose that causes 90% inhibition of a solid tumor
TMS	tetramethyl silane

CHAPTER-I

CHAPTER -I

Introduction & Review of Literature

1.1 : Introduction :

The chemistry of platinum has been studied widely for over two centuries. Most of the work published through 1971 is nicely summarized by F. R. Hartley **[1]**. Platinum exhibits oxidation states ranging 0 to 6. The divalent states is the most common in aqueous systems and forms compounds with members of nearly every group in the periodic table. In this chapter the aqueous solution chemistry of divalent platinum, especially in the presence of biological molecules, is discussed. A comprehensive review of even the most recent literature has not been attempted, owing to space limitations.

The chapter is organized in the following manner. The first portion covers broad principles of aqueous platinum (II) chemistry. An introduction to the divalent oxidation state and to basic thermodynamic and kinetic principles of platinum (II) reactions is followed by a more comprehensive examination of aqueous substitution reactions including both hydrolyses and the effects of salts on aqueous equilibria. Biological applications are discussed in the latter portion of the chapter. One section each is devoted to discussing the known and suspected platinum binding sites on biopolymers, the platinum blues, and the analytical methods used to detect platinum in its interactions with biological molecules.

(a) Introduction to Platinum (II) Chemistry :

Platinum (II) has the electronic configuration $[Xe]4f^{14}5d^{8}$. Although both five- and six-coordinate compounds are known,

the majority of its compounds exhibit coordination number 4 with square planar geometry **[1-5]**. The stability of square coordination for a d^8 configuration is apparent upon examination of the relative energies of the d orbitals in different coordination environments, as calculated according to crystal field theory (Fig.1.1) **[6]**. The orbital splittings shown in Fig.1.1 are only approximate for a given coordination environment, however, since the actual orbital energy differences depend upon the nature of both the metal and its ligands. For example, although comparison of five-coordinate environments for a d^8 system (Fig.1.1) indicates a slight advantage for square pyramidal over trigonal bipyramidal geometry, the known penta-coordinate platinum (II) compounds are trigonal bipyramidal **[1,3-5]**. These five-coordinate compounds all contain at least one ligand capable of accepting π-electron density from the metal, thereby lowring the energy of the metal's π-donating d orbitals. This π interaction renders the trigonal bipyramidal configuration more stable than the square pyramidal one **[1]**. Both four- and five-coordinate platinum (II) complexes are diamagnetic.

Spuare planar platinum (II) complexes are relatively kinetically inert, which is a feature of considerable importance in their chemistry. Although the radius of square planar platinum (II) is exactly the same as that of the d^8 palladium (II) ion, compounds of the latter react up to 10^6 times faster than the corresponding platinum (II) complexes **[1,2]**. The major electronic difference between the two elements is the existence

of 4f electrons for platinum. Because of the effective nuclear shielding of these 4f electrons, the divalent ions are the same size. The 5d orbitals of platinum are more extended spatially than the 4d orbitals of palladium, however, inhibiting axial coordination and retarding the rate of substitution reactions. Platinum (II) complexes are, therefore, more inert.

Platinum (II) forms stable compounds with both σ and π-electron donating ligands. Differences in the nature of the electron donation and in the ability of a ligand to accept metal π-electron density influence the thermodynamic and kinetic behaviour of platinum (II) complexes **[1,2,7,8]**. The ligands are generally anionic or neutral and the list of donor atoms encompasses nearly every non-metallic element. Because compounds formed with the heavier non-metallic elements are generally more stable, platinum (II) is classified as a 'b' type metal **[1,9,10]**. Class 'a' metals form their most stable compounds with the first member of a group : nitrogen, oxygen, fluorine. Class 'b' metals form their most stable compounds with the heavier members of the chemical family. Although the usual order for stability of a class 'b' metal with donor atoms is S ~ C > I > Br > Cl > N > O > F **[9,10]**, the environment of the donor atom must be considered. In the case of platinum (II), thioether (R_2S) and thiolate (RS^-) complexes are much more stable than those with R_2O and RO, whereas, H_2O and ROH complexes are more stable than those with H_2S and RSH. Complexes containing OH^- and SH^- are of comparable stability **[1]**. Classification as a class 'b' metal does

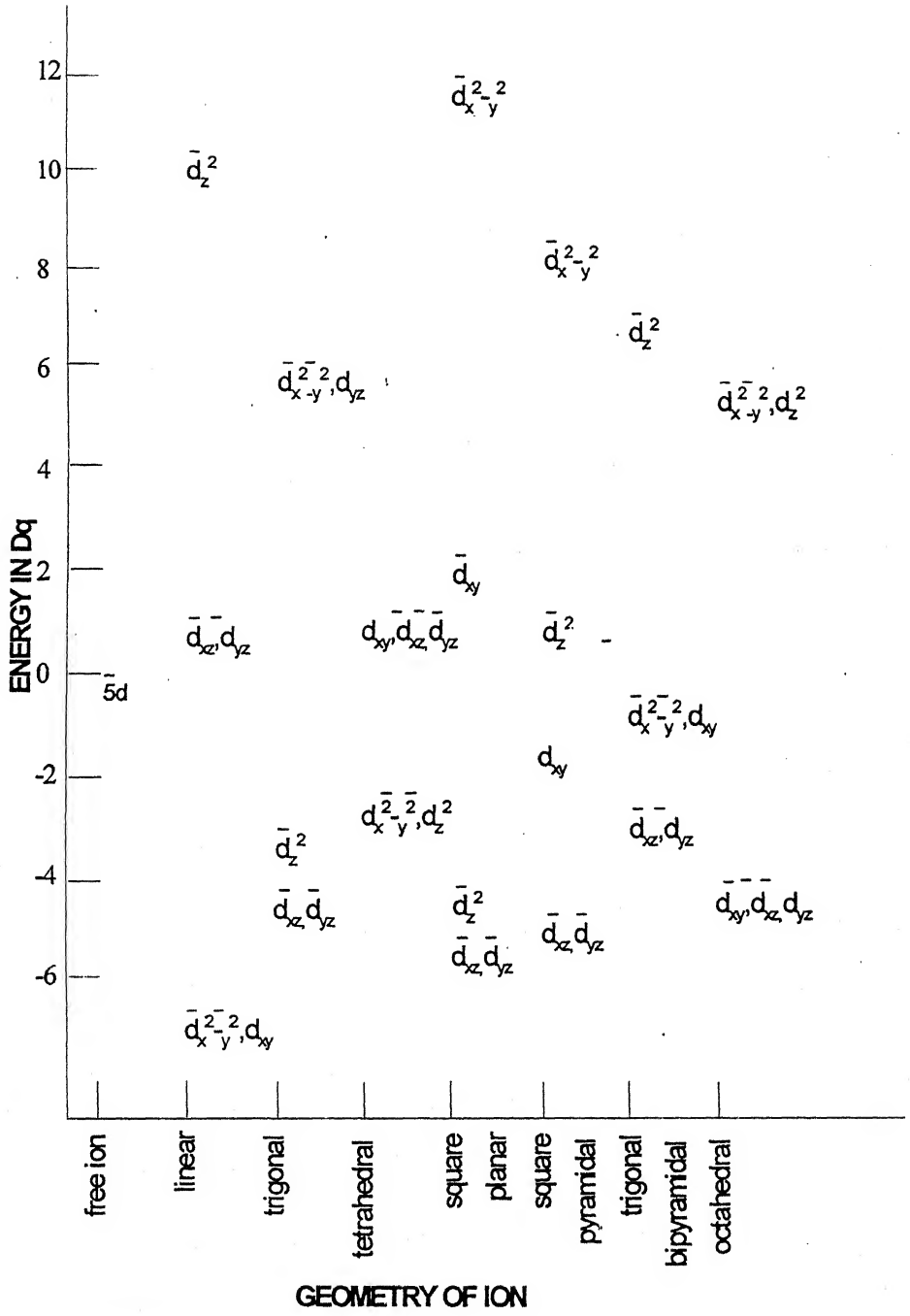


Fig.1.1: Relative energies of metal d orbitals in various coordination environments. Splittings are according to Ref.**[6]**.

not, therefore, preclude the existence of stable complexes with the first member of a chemical group, nor does the classification imply anything about the kinetic properties of a particular metal complex.

Ligand donor atoms with the appropriate orbital geometry and available electron density can bridge two or more platinum atoms, forming dimeric or polymeric species. For one series of anionic ligands, the tendency to form bridges was found to decrease as PR_2^- > SR^- > $R_2PO_2^-$ > I^- > Br^- > Cl^- > RSO_2^- > $SnCl_3^-$ **[2,11]**. In aqueous solution of moderate or high pH, the hydroxide ion often serves as a bridging ligand. Hydroxo-bridged complexes are discussed in some detail in Sec.1.3.d.

Apart from ligand-bridged systems, square planar platinum (II) compounds such as [(en)$PtCl_2$] form polymeric species in the solid state through axial metal-metal stacking interactions **[12]**. The polymeric, stacked structure of the platinum blues appears to be maintained even in solution **[13]**. Platinum compounds that undergo stacking interactions were recently reviewed **[14]**. The majority of these complexes display unusual conductivity properties **[12-15]**.

1.2 : Thermodynamic and Kinetic Principles :

(a) Thermodynamic Stabilities :

The thermodynamic stability of a coordination complex may be expressed either in terms of a series of stepwise formation constants, K_1, K_2, ..., K_n, where n is the number of

maximum coordination, or in terms of the overall formation constant β_n, defined in Eq. (i).

$$\beta_n = \prod_{i=I}^{N} K_n \qquad \text{(i)}$$

The stepwise formation constants for a square planar platinum (II) system and the corresponding chemical processes are shown in Fig. 1.2. Table 1.1 gives the overall formation constants of platinum (II) complexes formed from some common ligands in aqueous solution. As expected for a class 'b' metal, $\beta_4(I^-) > \beta_4(Br^-) > \beta_4(Cl^-)$. β_4 values for $[PtCl_4]^{2-}$ and $[PtBr_4]^{2-}$ were recently determined at 25^0C in a 0.5 to 1.00 M $HClO_4$ medium to be 9.77×10^{13} and 1.3×10^{16}, respectively **[16]**. Although the values at this higher ionic strength are less than those reported in Table 1.1, the order of the stabilities remains the same.

The relative affinities of various ligands for Pt (II) may also be ascertained by examining equilibrium constants for reversible ligand substitution reactions. Table 1.2 contains some of these data. Information from Tables 1.1 and 1.2 reveals that the relative affinity of ligands for Pt (II) decreases as $CN^- > NH_3 \sim OH^- > I^- > SCN^- > Br^- > Cl^- >> F^- \sim H_2O \sim$ MeOH. Thermodynamic stability is always measured relative to other components of the system. A complex will be thermodynamically stable depending upon the nature and concentration of other potential ligands. The stability of

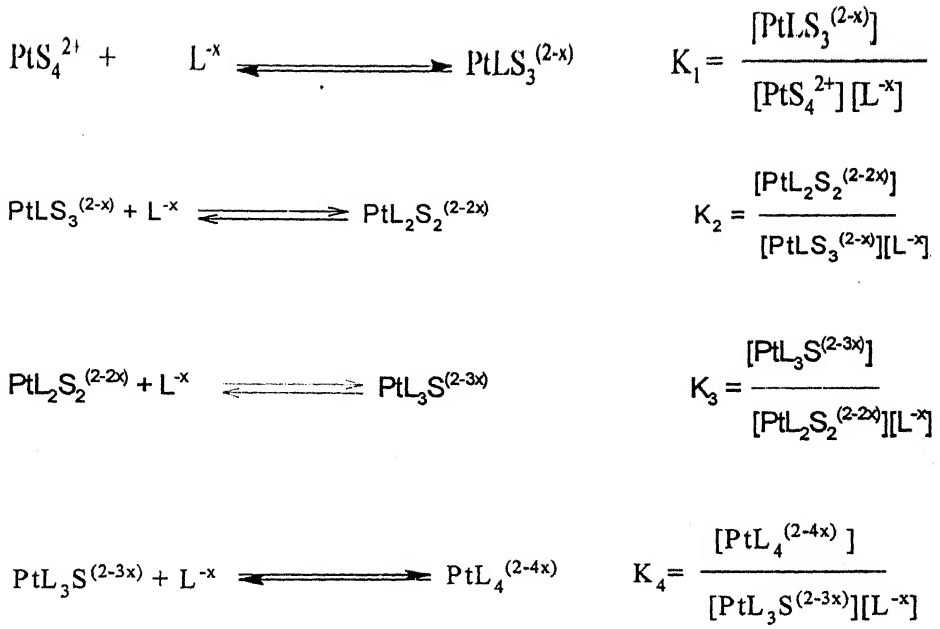


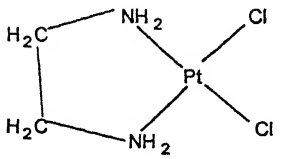
Fig.1.2. Platinum (II) stepwise formation constants. S represents the solvent, and L^{-x} is the ligand .

complexes in aqueous systems, where water is a potential ligand present in high concentration, is discussed in the next section. Moreover, square planar complexes often can exist as cis and trans isomers. Although the formation of the initial complex is kinetically controlled, the trans isomers are generally more stable than the corresponding cis compounds. For example, cis-$[Pt(NH_3)_2Cl_2]$ isomerizes to the trans species with an estimated ΔH^0 of isomerization of - 3.0 kcal mol^{-1} **[10]**.

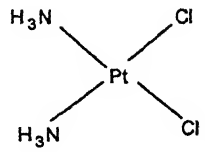
(b) The Chelate Effect :

A complex that contains a chelated ligand is generally more stable than a similar complex having no chelate rings, especially if the ring size is 5 or 6. The enhanced stability of chelated systems is called the chelate effect. The chelate effect

has both entropic and enthalpic origins **[2,10]**. Because of the chelate effect, dichloroethylenediammineplatinum (II) is expected to be more stable than cis-dichlorodiammine platinum (II), both of which are shown below.



[(en)PtCl2]



cis-[Pt(NH3)2Cl2]

(c) Redox Chemistry of Platinum (II) :

The stability of platinum (II) with respect to disproportionation to Pt (0) and Pt (IV) or to oxidative addition is of interest. Table 1.3 contains some platinum reduction potentials. Whereas, $[PtCl_4]^{2-}$ is stable to disproportionation [Eq. (ii)] at 1 M concentrations and 25°C, it is unstable at 60°C. The equilibrium constant for Eq. (ii) at 60°C in the presence of 3 M HCl is 50 M^{-1} versus 0.21 M^{-1} under standard conditions.

$$2PtCl_4^{2-} \rightleftharpoons Pt(0) + PtCl_6^{-2} + 2Cl^- \qquad \text{(ii)}$$

Oxidative addition reactions such as that shown in Eq. (iii) may occur with even the most stable of platinum (II) complexes,

$$2Pt(CN)_4^{2-} + I_2 \rightleftharpoons \text{trans-}Pt(CN)_4I_2^{2-} \qquad \text{(iii)}$$

β_4 (CN^-) = 10^{41}. The equilibrium constant reported for this reaction at 25^0C in 0.50 M $HClO_4$ is 1.29 x 10^4 M^{-1} **[18]**. Table 1.4 lists some additional reactions for which thermodynamic data are not available.

(d) Trans and Cis Influences :

The trans and cis influences are two thermodynamic phenomena observed for square planar platinum (II) complexes. The trans influence **[21]** is the tendency of a ligand to weaken the bond trans to itself in the ground state of a metal complex. The cis influence is the analogous effect on a cis ligand. The experimental evidence and theoretical rationale for the trans influence have been thoroughly reviewed **[7]**. Most of the information supports the theory that a rehybridization of metal σ orbitals occurs, whereby, the ligand exhibiting a strong trans influence competes more effectively for the platinum 6s orbital than do the other ligands. The bond in the trans position is, therefore, weakened. The net transfer of electron density from ligand to metal is also of importance, however, and under certain circumstances, e.g., π bonding, may outweigh the degree of metal rehybridization.

The trans influence is revealed by comparing bond lengths determined by x-ray crystallography for similar compounds **[7,22,23]**; by nmr chemical shifts and coupling constants, since these parameters reflect the hybridization of a metal ion **[7,24,25]**; by infrared spectroscopy **[7]**; and in the case of complexes containing Pt-I bonds, by Mossbauer

parameters, which reflect both differences in s character and overall σ and π interactions in the metal-iodide bonds **[26]**. While the trans influence is partly dependent upon the total composition of a complex, it generally decreases as PF_3 > PEt_3 > C_2H_4 > CO > H_2S > NH_3 > H_2O for neutral ligands and H^- > SiH_3^- > CN^- > CH_3^- > I^- > Cl^- > OH^- > NO^-_2 for anionic ligands **[27]**. A comparison of equilibrium constants corresponding to ground-state energy differences of Pt (II)-DMSO complexes showed the trans influence to vary as NH_3 > DMSO ~ C_2H_4 > Br^- ~ Cl^- ~ H_2O with factors relative to H_2O of 10(NH_3) : 4(DMSO) : 3(C_2H_4) : 1(Br^-, Cl^-) **[28]**. In the case of σ-bonding ligands, there appears to be a correlation between the trans influence and the kinetic trans effect discussed below **[7,22]**. For π-bonding ligands such as ethylene, however, there is no correlation between these two phenomena, presumably owing to differences in their origin.

There is some disagreement over the relative importance of the cis influence in square planar complexes. Molecular orbital calculations **[27,29]** indicate the cis influence to be of comparable magnitude to the trans influence. For a series of Pt (II) iodide complexes it was calculated that the cis influence is highest for ligands having empty, low-lying d orbitals **[27]**. The order of cis influence was found to be PH_3 > H_2S > C_2H_4 > NH_3 > H_2O > CO for $PtLI_3$, when L is neutral, with the same order for trans-PtI_2L_2, except that CO > H_2O. When L is anionic the order decreases as SiH^-_3 > CH^-_3 > OH^- > I^- > CN^- > H^- > Cl^-

> NO_2^- for $PtLI_3^{2-}$ and SiH^-_3 > CH^-_3 > OH^- > I^- > CN^- > H^- > Cl^- > NO_2^- > for trans $PtLI^{2-}_3$ and SiH_3^- > CH_3^- > I^- > H^- > OH^- > Cl^- > NO_2^- for trans-$PtI_2L_2^{2-}$. The experimental evidence for the cis influence is much less than that for the trans influence **[7]**, however. The cis influences of Pt(II)-DMSO compounds were found to be NH_3 > H_2O ~ Cl^- ~ Br^- > C_2H_4 ~ DMSO with factors relative to H_2O of 1-2 (NH_3) : 1 (Br^-, Cl^-) : 0.3 (C_2H_4) : 0.1 (DMSO) **[28]**. These values are all lower than the corresponding trans influence factors.

(e) Kinetics of Substitution Reactions :

Square platinum (II) complexes are, comparatively speaking, kinetically inert. Their substitution reaction kinetics have been widely studied and these studies have been extensively reviewed **[1,10,30,34]**. The complexes are susceptible both to nucleophilic attack, owing to the positive charge on the metal centre, and to electrophilic attack, owing to considerable d-electron density of the metal. Substitution reactions, such as that shown in Eq. (iv), Where Y is the entering group and X the leaving group, occur with retention of

$$PtL_3X^n + Y^y \longrightarrow PtL_3Y^n + X^x \qquad \text{(iv)}$$

configuration (cis or trans). The reactions generally follow the rate law given in Eq. (v) where k_1 is the first-order rate constant reflecting a solvolytic pathway and k_2 is the second order rate constant appropriate for a bimolecular substitution process. In general $k_2 >> k_1$.

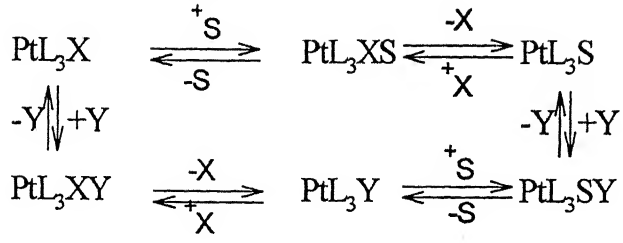
$$\text{Rate} = (k_1 + k_2[Y])(\text{complex}) \qquad (v)$$

Although the evidence is indirect, the favoured reaction mechanism for substitution is associative, involving a five-coordinate intermediate as shown in Fig.1.3. In the figure, S represents the solvent. The associative mechanism is supported by kinetic data in which the complex charge, solvent, and bulk of the side groups have been varied. The evidence is briefly summarized below. A more thorough review of the literature is available elsewhere **[1,10,30,35]**. For a series of reactions in which the entering and leaving groups are the same, a variation in charge on the complex has a very small effect on the first-order rate constant, even though the entering group may be charged. For example, for the platinum (II) ammine chloride complexes $[PtCl_4]^{2-}$ to $[Pt(NH_3)_3Cl]^{+}$ in aqueous media, k_1 only varied from 0.62×10^{-5} to $9.8 \times 10^{-5} sec^{-1}$ **[1]**. The value of k_1 changes with changes in solvent. For the reaction shown in Eq. (vi), k_1 changed from 3.5×10^{-5} to 38×10^{-5} sec^{-1} upon going from H_2O to DMSO **[1]**. A sterically hindered system results in a sharp decrease in

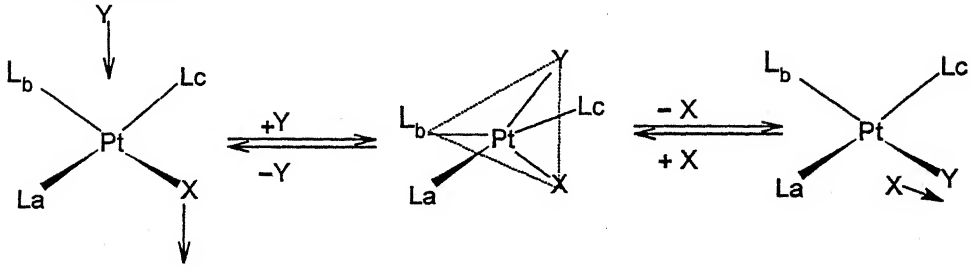
$$trans\text{-}Pt(py)_2Cl_2 + 2R_4N^{36}Cl \longrightarrow trans\text{-}t(py)_2{}^{36}Cl_2 \qquad (vi)$$

reaction rate; 2-substituted pyridines are much less reactive towards square planar complexes than are 3- and 4-substituted derivatives **[34]**. The value of k_2 depends on the nature of the entering ligand, Y. For the reaction shown in Eq. (vii). k_2 changed form 0 to 4300×10^4 for $Y = OH^-$ and SCN^-, respectively**[1]**, in aqueous solution at 25°C. The volumes

(i) Possible Pathways



(ii) Geometric Configurations for the Direct Bimolecular Reaction



(iii) Reaction Coordinate Energy Profile for the Geometric Configurations of (b)

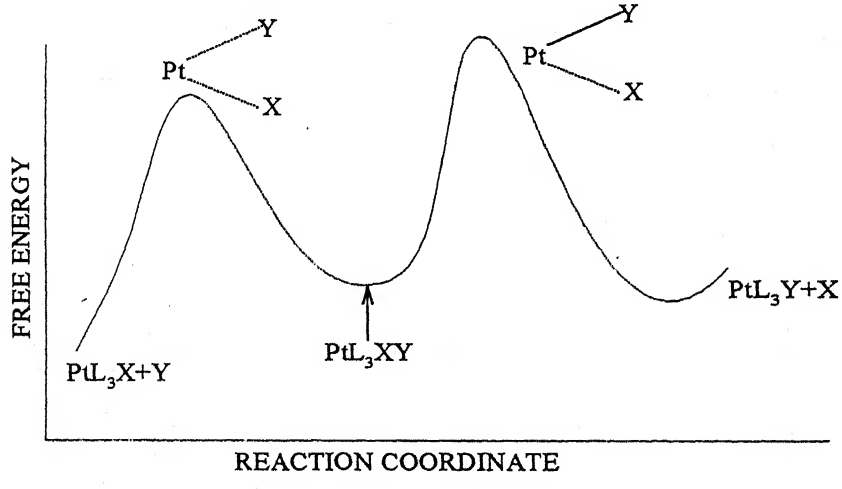


Fig.1.3 Associative reaction mechanism for Pt (II) substitution reaction.

$$Pt(dien)Bt^{+} + Y \longrightarrow Pt(dien)Y^{2+} + Br^{-} \qquad (vii)$$

of activation for these reactions are very negative. Negative values for volumes of activation are indicative of bond formation in the transition state.

The intermediate of the associative reaction mechanism is thought to be a trigonal bipyramidal [Fig.1.3(b)], which is consistent with the observed retention of configuration. Moreover, all known five-coordinate platinum (II) complexes are trigonal bipyramids, at least in the solid state **[1,3-5]**, and a five-coordinate intermediate in an Au (III) substitution reaction was actually isolated and shown to have this geometry **[36]**. Au (III) complexes are also square planar. Theoretical calculations using both ligand field **[37,38]** and molecualr orbital **[8]** theory support an associative mechanism and a trigonal bipyramidal (tbp) transition state geometry. The molecular orbital analysis used a tbp intermediate and generated a double-humped potential energy curve as shown in Fig. 1.3 (iii).

The dependence of the bimolecular reaction rate on the nature of the entering ligand has led to the definition of a nucleophilicity scale for platinum (II) complexes **[39]**. The reactivity of various ligands is related to their polarizability and to the amount of ionic character and σ versus π interactions in their binding to the metal centre. The nucleophilicity scale is based on the reactivity of a ligand towards the complex trans-$[Pt(Py)_2Cl_2]$ in methanol. The

nucleophilicity of a ligand is given the symbol n^0Pt, defined in Eq. (viii), where k_y and k^0s are the second-order rate constants for the bimolecular and for the

$$n^{o}_{Pt} = \log \frac{k_y}{K_s^0} \qquad \text{(viii)}$$

solvolytic reaction pathways, respectively ($k^0_s \equiv k_1$, [MeOH]). Table 1.5 contains values of n^0_{Pt} for a variety of ligands. These data can be extended to other ligands if the nucleophilic discrimination factor s, defined by Eq. (ix), is known.

$$sn^0_{Pt} = \log \frac{k_2^y}{K_s^0} \qquad \text{(ix)}$$

The nucleophilic discrimination factor for $[Pt(en)Cl_2]$ in water is 0.64; the value given to trans-$[Pt(py)_2Cl_2]$ in MeOH is 1.00 **[39(a)]**. The n^0_{Pt} values are calculated from kinetic measurements; they have not been meaningfully correlated to any other properties of the ligand.

The order given in Table 1.5, however, is similar to the order of the kinetic trans effect, which depends upon the non-labile ligands in a complex. The kinetic trans effect is discussed in the following section.

The rate of a substitution reaction also depends upon the composition of the metal complex, in which both labile and non-labile ligands are influential. The leaving group, or labile ligand, has been found to affect the bimolecular rate constant k_2 in the order $NO^-_3 > H_2O > Cl^- \geq Br^- \geq I^- > N^-_3 > SCN^- > NO^-_2 >$

CN^- for a variety of complexes **[10,34]**. Moreover, for any given class of leaving groups, the more basic the ligand, the slower the reaction rate **[40].** Also of importance is the fact that the non-labile ligands can influence not only the rate of a substitution reaction, but also which labile ligand is the leaving group. The ligand of greatest importance is the one trans to a potential leaving group.

(f) Kinetic Trans and Cis Effects :

The kinetic trans effect, sometimes referred to as the labilizing trans effect, is measured by the effect on the reaction rate of the ligand T trans to the leaving group. The kinetic trans effect differs from the thermodynamic trans influence. The latter reflects the ground state of a complex, while the former involves a reaction transition state or intermediate, assumed to have a trigonal bipyramidal structure. The trans influence helps to determine which ligand will be the leaving group; the trans effect is of importance in determining how quickly the designated ligand will depart. These phenomena may or may not be related. A compilation of kinetic data from numerous sources has produced the following order of trans labilizing ability : olefines $\simeq$ No $\simeq$ CO $\simeq$ CN^- > R_3Sb > R_3P $\geq$ R_3As $\simeq$ H^- $\simeq$ $SC(NH_2)_2$ > CH^-_3 > $C_6H^-_5$ > SCN^- > NO_2^- > I^- > Br^- > Cl^- > py $\geq$ NH_3 > OH^- > H_2O. DMSO has also been found to have a large trans effect **[17,28]**; in one report the labilizing ability was found **[28]** to be $H_2O < NH_3 < Cl^- < Br^- <$ DMSO $< C_2H_4$ as $1 < 200 < 330 < 3000 < 2 \times 10^6 < 10^{11}$.

There is also a kinetic cis effect which is generally of lesser magnitude than the corresponding trans phenomenon **[28,34]**. The cis effect ligand order is C_2H_4 < Br^- ~ Cl^- < NH_3 ~ H_2O < DMSO as 0.05 < 0.3 ~ 0.4 < 1 ~ 1 < 5 **[28]**. Interestingly, it was found that the less basic the cis ligand, the larger the cis effect, that is, the faster the reaction rate **[41]**. For a series of amines cis to an entering amine ligand in a substitution reaction of cis [Pt(DMSO)(am)Cl_2] in methanol, the basicity of the entering amine did not affect the reaction rate but the basicity of the cis ligand was found to affect K_2 in the manner shown in Eq. (x), where C is a constant. It should be noted,

$$\log k_2 = -0.4pK_a + C \qquad (x)$$

however, that the order assigned to ligands for either the cis or trans effect depends in part upon the nucleophilicity of the entering group, n°_{Pt} **[34]**.

There have been several attempts to elucidate the factors contributing to square planar substitution reaction kinetics. Kinetic parameters in close agreement with experimental values for reactions of the [$Pt(NH_3)_x(H_2O)_yCl_{4-x-y}$] system have recently been generated **[8]**. The bond-making process was found to be most often rate-determining. The rate of reaction increases, owing to a lowering of the initial barrier to reaction intermediate formation, with increasing σ-donor strength of the entering ligand, π acceptor orbitals on the entering ligand, good interactions with (n+1)s,p orbitals on the metal by the entering ligand, entering ligand softness, decreasing σ-donor

strength of the trans ligand, decreasing σ-donor strength of the leaving ligand, and increasing σ-donor strength of the cis ligands. The trans effect of a ligand was also found to increase with decreasing σ-donor strength of the ligand T, increasing π-acceptor strength of T, good interaction of T with (n + 1)s,p orbitals on the metal, and increasing class 'b' character of T.

(g) Kinetics Related of Chelation :

Also of interest to the substitution reaction kinetics of platinum (II) are the kinetics of chelation. Studies have been made of the kinetics of ring closure of trans-$[Pt(enH)_2Cl_2]^{2+}$ **[42]**, both ring-closing and -opening for a series of cis-$[Pt(DMSO)(amH)Cl_2]^{2+}$ complexes where amH is a protonated diamine of varying length **[43]**, and rates of ring closure involving a second chelate ring using triamines **[44]**. In all cases the ring-closing rate is extremely fast compared to the addition of a monodentate amine. For example, the rate of ring closure for ethylenediamine in the DMSO complex is 1.5×10^4 sec^{-1}, compared with 3.79 sec^{-1} for the addition of a second cyclohexylamine ligand **[43(a)]**. The rate of ring opening of ethylenediamine is only slightly less than substitution of a cyclohexylamine species, 1.0×10^{-4} sec^{-1} versus 1.5×10^{-4} sec^{-1} **[43(a)]**. These data suggest that the chelate effect is a combination of a larger than expected ring-closing rate together with a smaller than expected ring-opening rate.

Complexes containing aromatic chelate ring systems such as $[Pt(bipy)Cl_2]$ and $[Pt(terpy)Cl]^+$ react much faster,

respectively, than $[Pt(py)_2Cl_2]$ or $[Pt(dien)Cl]^+$ **[45]**. This result is ascribed to the high trans labilizing effect of ligands such as bipy and terpy **[10,46]**.

Bridged binuclear complexes also can react like chelated systems. For monoatomic bridges such as halides, the reaction rates are from 10^2 to 10^3 times faster than corresponding monomeric systems, reflecting the strain expected in a four-membered ring **[10]**. The rate law usually follows the same form as mononuclear complexes, however, with a nucleophile needed to open the bridge, regardless of the strain in the parent compound. Additionally, ligand exchange sometimes proceeds through a dimeric intermediate. Halide exchange in cis-$[(NH_3)_2PtX_2]$ systems is catalyzed by the presence of $[PtX_4]^{2-}$, X = Cl^- or Br^- . A doubly bridged intermediate is proposed **[47]**.

(h) Additional Kinetic Factors :

Although the majority of substitution reactions are associative, in some cases a dissociative mechanism may be of importance. Anomalous reactions, not obeying the rate law of Eq.(v), and dissociative reactions have been summarized **[35,48]**. There is considerable evidence for a dissociative mechanism, especially when the metal is sterically hindered from axial coordination **[34,35,49]** or undergoes photosubstitution **[50]**. Moreoveer, platinum (II) complexes catalyze substitution in platinum (IV) systems **[51]**; this topic has been recently reviewed **[52]**.

In addition to substitution reactions, both free-ligand catalyzed and spontaneous cis-trans isomerizations occur for platinum (II) complexes. The mechanism of isomerization, long believed to be associative, is now thought to depend upon both the solvent system and the complex; it may be either associative or dissociative **[34,35,53]**.

Reactions involving coordinated ligands of platinum (II) complexes also occur. For example, chelated products form in the reaction of amines with monodentate isocyanide complexes **[54]**, and water is postulated to add to coordinated aromatic chelate rings **[55]**. The latter reaction will be discussed in Sec. 1.3.e.

1.3 : Hydrolysis and Aqueous Substitution Chemistry :

(a) Effects of Buffers and Salts on Complex Stabilities :

Many buffer-salt systems, both in vitro and in vivo, contain potential ligands, e.g., Cl^-, phosphate, HCO^-_3, citrate, acetate, $C_2O_4^{2-}$, OH^-, or ammonia, the concentrations of which can affect the nature of a platinum (II) complex in solution. In aqueous media, of course, water is present in highest concentration. Although water is a potential ligand, it is also an excellent leaving group and hydrolysis products can be suppressed in the presence of better nucleophiles in sufficiently high concentration. The chloride ion is especially effective in inhibiting hydrolysis reactions. Water is ~70 times faster a leaving group than Cl^- in some amine systems **[56]** and ~40 times faster in a DMSO complex **[43(a)]**.

In a chloride medium comparable to blood plasma ($[Cl^-]$ ~ 103mM) and at physiological pH and temperature (pH = 7.4, T ~ 37^0C), the species present in a solution containing $[(en)PtCl_2]$ are $[(en)Pt(H_2O)_2]^{2+}$: $[(en)Pt(OH)_2]$: $[(en)Pt(H_2O)(OH)]^+$: $[(en)Pt(H_2O)(Cl)]^+$: $[(en)Pt(OH)(Cl)]$: $[(en)PtCl_2]$ in the ratio 0.00134 : 0.033 : 0.053 : 1 : 1 : 37.3 **[56]**. In a low-chloride-ion medium, compsarable to the cytoplasm of a cell ($[Cl^-]$ = 4 mM, pH = 7.4), the same species have the ratio 0.0345 : 0.086 : 1.38 : 1 : 1 : 1.45. These results suggest **[56]** that $[(en)PtCl_2]$ would be about 10 times as reactive in the cell than in the plasma. Using data found in Tables 1.6 and 1.8, and assuming the pK_a of the aquochloro complex of the cis-diammine is the same as that attributed to the ethylenediamine compound, 7.4, the species derived from cis-$[(NH_3)_2PtCl_2]$ would be diaquo : dihydroxo : aquohydroxo : aquochloro : hydroxochloro : dichloro in the ratio 0.0178 : 1.41 : 1.12 : 1 : 1 : 24 in the plasma and 0.46 : 3.63 : 2.90 : 1 : 1 : 0.915 in the cytoplasm. Comparable data are not available for complex formation with buffers or salts composed of other potentially coordinating ligands. It may be noted, however, that acetate ion is a poorer nucleophile towards platinum (II) than is chloride (Table1.5) and thus is a poorer entering group. On the other hand, ammonia is a slightly better nucleophile than chloride.

Perchlorate and sulfate ions are considered to be non-coordinating for platinum (II) and nitrate is a better leaving group than water **[10,34]**. Buffer systems composed of these

anions are non-coordinating and may be used when the integrity of a complex containing a labile ligand is desired. Hydrolysis reactions and kinetic studies of platinum (II) complexes are often carried out in acidic perchlorate media.

(b) Hydrolysis of Platinum (II) Complexes : Substitution Reactions :

The absence of better nucleophiles or poorer leaving groups than water in aqueous systems facilitates the investigation of hydrolysis reactions of platinum (II) complexes. Table 1.6 contain equilibrium constants for a number of hydrolysis reactions and Table 1.7 gives some typical rate constants. Although Table 1.6 reveals that cis-$[(NH_3)_2PtCl_2]$ is less stable to hydrolysis than $[(en)PtCl_2]$, Table 1.7 shows that the latter compound reacts ~ 35% faster than the former. That cis-$[(NH_3)_2PtBr_2]$ is more stable to hydrolysis than the chloride compound (Table 1.6) is expected for a class 'b' metal. The rates of reaction for trans-DMSO complexes (Table 1.7) reflect the kinetic trans effect discussed earlier while the rates of halide anation of Cl^- < Br^- (Table 1.7) are expected from the n^o_{Pt} values of Table 1.5.

The hydrolysis of $[PtCl_4]^{2-}$ to form $[PtCl_3(H_2O)]^-$ proceeds faster in the presence of Zeise's anion, $[PtCl_3(C_2H_4)]^-$, than in its absence **[60]**. The catalyst is thought to be trans-$[Pt(C_2H_4)Cl_2(H_2O)]$, since the increase in the rate of hydrolysis of $[PtCl_4]^{2-}$ parallels an increase in the concentration of the

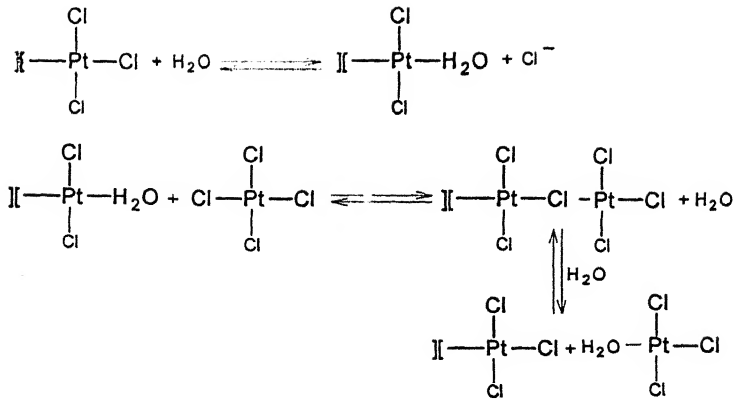


Fig.1.4. Mechanism proposed for the catalysis of $[PtCl_4]^{2-}$ hydrolysis by Zeises's anion, omitting charges on platinum-containing species (Adapted from Ref . **[60]**).

trans-$[Pt(C_2H_4)Cl_2(H_2O)]$. The mechanism proposed **[60]** involves the chloride- bridged intermediate shown in Fig. 1.4. The suggestion that decomposition of the bridge results in reformation of Zeise's anion is interesting in view of the much larger trans-labilizing ability of C_2H_4 compared with that of chloride ion.

(c) Acidity of Coordinated Water :

Platinum aquo complexes are weak acids with reported pK_a values ranging from $\geq$ 2.5 for $[Pt(H_2O)_4]^{2+}$ to 7.6 for $[(en)Pt(H_2O)(OH)]^{+}$. The pH_a values of some platinum hydrolysis products are given in Table 1.8. The greater the trans influence of the ligand trans to the water molecule undergoing deprotonation, the less tightly bound is that water molecule and the higher the expected pK_a of the complex. For the complexes in Table 1.8, the pK_a values increase with H_2O <

amines < Cl^- in the trans position as predicted from the trans influence series.

(d) The Hydroxide Ion as Ligand :

The result of deprotonating coordinated water is the formation of a hydroxo complex. Whereas, the water molecule is an excellent leaving group, hydroxide ion is not. The affinity of hydroxide for platinum (II) is approximately that of ammonia. Substitution of coordinated hydroxide occurs after protonation to form water. Moreover, a platinum (II) bound hydroxide ion is still a good nucleophile, and it may act as a bridging ligand. Bridged hydroxide complexes are stable towards protonation and thus, exceedingly inert to substitution.

Although $[(en)Pt(OH)_2]$ appears to be indefinitely stable at high pH, the addition of one equivalent of hydroxide to a 6 mM solution of $[(en)Pt(H_2O)_2]^{2+}$ when left at ambient temperature for a few days produces a stable, unreactive product with no titratable group, assumed to be the dimeric $[(en)Pt(OH)_2]^{2+}$ **[56]**. A variety of hydroxobridged diammine complexes have been isolated as crystalline solids **[64]**. The first of these was $[(NH_3)_2Pt(OH)_2(NO_3)_2]$, an air-stable compound having nearly perfect D_{2h} symmetry obtained from a solution of pH = 6.44 **[64(a)]**. The infrared stretching band at $1040cm^{-1}$ shifts upon deuteration and is assigned to the bridging hydroxo ligand . A crystalline carbonate analog with four formula units per unit cell is also known **[64(b)]**. The stacking of this compound is

believed to be stabilized by hydrogen-bonding interactions between formula units rather than metal-metal interactions. Both complexes are stable over a large pH range, from ~ pH 2-11. Cyclic trimeric species with formulae $[(NH_3)_2Pt(OH)]_3(SO_4)_3.6H_2O$ **[64(c)]** and $[(NH_3)_2Pt(OH)]_3(NO_3)_6$ **[64(d)]** have also been characterized by x-ray crystallography. Crystalline cis-$[(NH_3)_2Pt(NO_3)_2]$ has also been isolated from a cis-$[(NH_3)_2Pt(H_2O)_2]^{2+}$ nitrate solution at pH 2 **[65]**. Between pH 4 and pH 7, the predominant species in a concentrated solution of cis-$[(NH_3)_2Pt(H_2O)_2]^{2+}$ are thought to be the dimeric and trimeric hydroxo-bridged complexes.

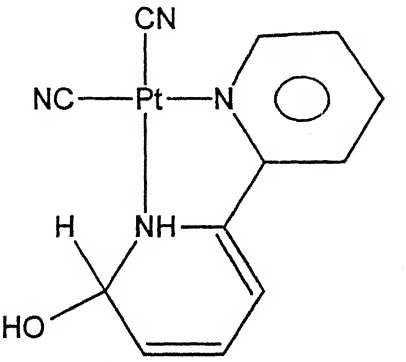
(e) Proposed Hydrolysis of Coordinated Ligands :

Water not only reacts as a nucleophile towards platinum (II), but also towards aromatic carbon atoms α to coordinated nitrogen. A covalently hydrated complex $[(bipy)Pt(CN)_2].H_2O$ with the structure shown in Fig.1.5(i), has been proposed **[55(a)]**. The anhydrous material, a red compound, has an nmr spectrum at 100^0C that is comparable to that of $[(bipy)PtCl(H_2O)]$. At 25^0C, the complex is a yellow material with an nmr spectrum quite different from that of the red species, showing two separate signals assigned to the protons on the two differing α carbons on the basis of $^{195}Pt(I = 1/2)$ coupling.

The hydroxide ion may also interact with coordinated pyridine rings as shown in Fig.1.5 (ii). The complex $[(bipy)_2Pt]^{2+}$ and its 5,5′-dimethyl analog undergo reversible electronic and

nmr spectral changes as a function of pH **[55(b), 55(c)]**. The pK_a of the unsubstituted bipyridyl complex is 9.0 while that of the 5,5′-dimethyl analog is 9.5. The complex ions $[(bipy)Pt(NH_3)_2]^{2+}$, $[(bipy)Pt(py)_2]^{2+}$ and $[(py)_4Pt]^{2+}$ do not exhibit these effects nor does the spectrum of $[(bipy)Pt(OH)_2]$ correspond to those of the bis-bipyridyl compounds. The

(i) $[(bipy)Pt(CN)_2].H_2O$



(ii) $\{[(bipy)_2Pt].OH\}^+$

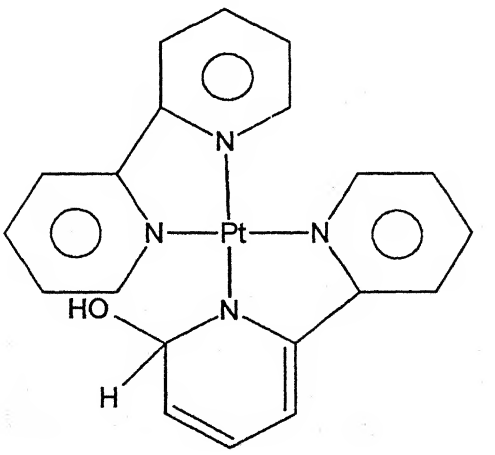


Fig.1.5. Proposed structures of covalently hydrated complexes (From Ref. **[55]**).

complex [(o-phen)$PtCl_2$] also exhibits reversible spectral changes with pH adjustments **[55(b)]**. These changes are believed to be a result of addition of hydroxide ion to the α-carbon atom of the aromatic amine. Hydroxide substitution and five-coordinate complexes have been ruled out on spectral grounds, as has formation of a conjugate base by proton abstraction from the coordinated ligand, since there is no exchange in alkaline D_2O **[135]**.

1.4 : Binding Sites in Biological Systems :

Biological systems have many donor atoms and binding sites for metal ions and platinum (II) is no exception. Because of the complexity of in vivo processes, however, most information about metal binding comes from studies of simpler, in vitro systems. The potential and known and/or suspected binding sites for platinum (II) with nucleic acids and proteins are separate topics and will be treated as such.

The identification of platinum (II) as a class 'b' metal suggests that biomolecules containing atoms of sulfur, carbon, and/or nitrogen that can serve as metal donors will form the most stable complexes with platinum (II). Metal binding will be kinetically controlled, at least initially, and the nucleophilicity of a potential ligand (Table 1.5), together with its leaving-group ability, are important considerations. The degree of structural accessibility of a potential ligand and its relative concentration are also important factors.

(a) Nucleic Acids : Potential Binding Sites :

The monomer units of polynucleotides consist of a phosphate diester, a pentose ring, and an organic base. Each portion of this nucleotide contains a binding site for platinum (II). The negative charge of the phosphate moiety is a source of electrostatic attraction to a positive platinum (II) ion. Moreover, the terminal oxygen atoms of the phosphate group are potential metal-ion donor ligands. Although the affinity of the class 'b' platinum (II) for class 'a' phosphate oxygen is quite low, the phosphate residue is geometrically situated so as to allow metal coordination without major disruption of the structure of the biopolymer. Additionally, these readily accessible, negatively charged species are present in relatively high concentration. In DNA there are two phosphate residues every 3.4 Å along the double helix **[66]**. Phosphate oxygen, however, is expected to be about as good a leaving group as nitrate oxygen, which has been shown to be a better leaving group than water **[10, 34]**.

The sugar residue, either ribose or deoxyribose, is a neutral entity containing a ring oxygen atom, which theoretically is a potential ligand. Ether oxygens, however, are known to be poor donor atoms in general and, in particular, have little or no affinity for platinum (II) **[1]**. Apart from the ring oxygen, the ribose (but not deoxyribose) ring carries a 2′-hydroxyl group, which may also coordinate to a metal. The affinity of alcoholic oxygen for platinum (II), however, is also quite low **[1]**. Although sugar residues are present in

concentrations equivalent to that of phosphates, nucleic acid tertiary structure is such that in polymeric duplexes the pentose oxygens are generally oriented away from the hydrophilic solvent, and thus 2′-hydroxyl groups are not as geometrically accessible as are the phosphate oxygens to metal ions in solution. The 3′- and 5′-oxygen atoms of the phosphodiester linkage are also potential ligands but are unlikely to bind strongly to platinum (II) both for steric and electronic reasons.

The organic bases of the nucleotides offer the best potential donor atoms for platinum (II) coordination. DNAs are generally composed of adenosine(A), cytidine(C), guanosine(G), and thymidine(T) monophosphates while RNAs contain uridine (U) instead of thymidine monophosphate. The adenine and guanine purine bases and cytosine each contain heterocyclic nitrogen atoms that can serve as metal ligands. These pyridine-like systems are expected to have a reasonably high nucleophilicity for platinum (II) (Table 1.5) and be relatively poor leaving groups. Although thymine and uracil, in their keto forms, have no pyridine-like nitrogen atoms at neutral pH, they do exist in the enolic forms of these nucleotides. In addition to the ring nitrogens, the purines and cytosine have exocyclic amine groups which, like that of aniline, could serve as a metal donor. It should be noted, however, that an exocyclic amine in a nitrogen heterocycle, especially when attached to an α carbon atom, is a poor nucleophile and thus less attractive as a ligand than the amine of aniline (Table 1.5).

All bases except adenine also have exocyclic oxygen atoms. In their keto forms these oxygen atoms, although not class 'b' ligands, could act as metal donors for platinum (II). Although the affinity for platinum should drop drastically once the oxygens are in their enolic form, the deprotonated enolic oxygen would be a good ligand. The potential for a heterocyclic nitrogen and an exocyclic group to chelate a metal should also be noted. Less common bases with sulfur substituted for exocyclic oxygen, such as 4-thiouracil and 6-thioguanine, are expected to be excellent ligands for platinum (II).

Although the organic bases in nucleic acids contain the best potential ligands for platinum (II), the tertiary structure of the biopolymer does not always render these binding sites geometrically accessible to the metal. The bases are buried in the hydrophobic portion of the biomolecule, and metal coordination will be accompained by changes in polymer tertiary structure. The concentration of any given base is also less than that of the phosphate or sugar moieties, although this factor is probably not too important.

(b) Binding Sites on Nucleic Acid Constituents :

Nucleic acid binding of heavy metals in general **[67]**, and of platinum (II) with DNA constituents in particular **[68]**, have been recently reviewed. The most definitive information is available for platinum (II) binding to nucleic acid constituents, i.e., the bases nucleosides, and nucleotides, rather than to DNA, RNA, or even oligonucleotides. Platinum (II) is known to

bind covalently to each of the bases. The binding sites observed under conditions of neutral or near-neutral pH are summarized in Table 1.9.

As may be seen in Table 1.9, only N-1 and N-7 of adenosine bind platinum (II), although the exocyclic amino group on C-6 was proposed to bind the metal when its pKa is lowered through methylation at N-1. The N-7 atom is the favoured site on guanosine in neutral (or acid) solutions and no evidence for chelation through O-6 has been found at any pH, either in the reported crystal structures or in Raman or Proton nuclear magnetic resonance spectroscopic studies of cis- and trans-diammineplatinum (II) with 5′-GMP and polyguanylate **[69]**. Platinum (II) also binds to the deprotonated N-1 position of 5′-GMP under slightly alkaline conditions, pH = 8.3, and Pt-GMP ratios > 1. In cytosine, the ring nitrogen N-3 is the preferred binding site. No data exist to support chelation through neighbouring exocyclic keto or amino groups. Uridine and thymidine also bind platinum (II) through N-3, the metal substituting for the hydrogen normally found at that position. Although uracil and thymine form polymeric, blue compounds which presumably arise from bridged chelation with cis-diammineplatinum (Sec.1.5), neither uridine nor thymidine appears to form mononuclear chelated compounds with platinum (II) at neutral pH **[70]**.

The relative order of nucleophilicity of the ribonucleotides towards cis- and trans-diammineplatinum (II)

was determined to be GMP > AMP >> CMP >> UMP at 25^{0}C and pH 7 **[71]**. No differences between the ribo- and deoxyribonucleotides are expected and thymidine monophosphate should have a nucleophilicity comparable to UMP. The kinetics of the reaction shown in Eq. (xi), where L is a nucleoside, were studied at 37^{0}C. The k_2 values for guanosine,

$$[^{14}C]\text{-}Pt(en)Cl_2 + L \xrightarrow{k_2} [^{14}C]\text{–}Pt(en)LCl^+ + Cl^- \qquad \text{(xi)}$$

7-methylguanosine, and adenosine were found to be 0.106, 0.024, and 0.006 M^{-1} sec^{-1}, respectively **[72]**. These data indicate that guanosine derivatives are more reactive than adenosine towards platinum (II), even when the N-7 position is blocked. Moreover, the difference between the rates of substitution of guanosine and its 7-methyl derivative clearly point to N-7 as the kinetically preferred binding site for platinum (II). The trans effect of guanosine has been reported to be less than that of chloride or bromide since cis-[Pt(guanosine)$_2$X$_2$] (with X^- = Cl^-, Br^-) has been isolated, but the trans isomer has not **[73]**. In contrast to the observed kinetic differences, the stability constants of the various nucleic acid constituents with platinum (II) are all reported to be of comparable magnitude **[74]**.

(c) Binding to Polynucleotides :

Many investigations of platinum (II) binding to DNA and RNA have been undertaken but in the majority of these studies the binding site of the metal has not been determined. One

major exception involves platinum binding to crystals of yeast $tRNA^{Phe}$; tRNA crystals soaked in a $[PtCl_4]^{2-}$ solution bind platinum in four specific sites, three of which were used along with other heavy atom binding sites to determine phases in the crystal structure of the tRNA **[75]**. Although the specific donor atoms on the tRNA have not yet been identified, they presumably could be, with further refinement of the structure. Additionally, trans-$[(NH_3)_2PtCl_2]$ has been shown to bind specifically to N-7 of the guanosine-34 residue of yeast $tRNA^{Phe}$ **[76]**. This residue is in the anticodon loop of the tRNA. Cis-$[(NH_3)_2PtCl_2]$ does not appear to bind similarly to tRNA.

No evidence of platinum (II) binding to the ribose ring, either to the ring oxygen or to the exocyclic hydroxyl groups, has been found in any investigation involving nucleic acids or their constituents. Neither has any evidence for convalent interactions with phosphate moieties of natural DNAs or RNAs been reported. However, a covalent platinum (II)-phosphate oxygen link has been identified by x-ray crystallography in solid $[Pt(en)(5'\text{-CMP})].2H_2O$ (Table 1.9) **[77]**. Quantitative binding of $[^{3}H]$ $[Pt(terpy)Cl]^{+}$ to the sulfur atom of $[^{35}S]$ poly (-$_S$AU), a polynucleotide prepared from adenosine 5'-0-(1-thiotriphosphate) and UTP, has also been reported **[78]**. Apart from these two cases, reported platinum (II)-nucleic acid interactions have been either electrostatic in nature or involved binding to the bases. There does not appear to be a unique base-binding site, however.

The extent of platinum (II) nucleic acid binding is a function of the composition of the metal complex employed, the base composition of the polynucleotide, the ionic strength and composition of the medium, and the time allowed for incubation. It has been shown through potentiometric titrations and ethidium fluorescence inhibition studies that, whereas, $[Pt(dien)Cl]^+$ and $[Pt(NH_3)_3Cl]^+$, each with only one labile ligand, bind DNA covalently at only one site, complexes with two or more labile ligands, e.g., cis- and trans-$[Pt(NH_3)_2Cl_2]$ and $[PtCl_4]^{2-}$, bind more than one site on DNA **[79-81]**. The latter complexes also cross-link DNA **[81]**. Kinetically inert compounds such as $[Pt(terpy)(HET)]^+$, HET = 2-hydroxyethanethiolate, and $[Pt(NH_3)_4]^{2+}$ do not bind DNA or RNA covalently **[79,80,82]**. The non-aromatic, non-planar kinetically inert compounds, e.g., $[Pt(NH_3)_4]^{2+}$ or $[Pt(py)_2(en)]^{2+}$, exhibit only electrostatic interactions, while the planar, aromatic compounds, e.g., $[Pt(terpy)HET]^+$ and $[Pt(bipy)(en)]^{2+}$, bind to double-helical nucleic acids by intercalation **[82,83]**.

The buoyant density of DNA containing bound cis-$[Pt(NH_3)_2Cl_2]$ is proportional to the G.C content of the biopolymer and depends upon its sequence **[84]**. The buoyant densities of platinum (II)-bound natural DNAs increase with G.C base pair content, whereas, the buoyant density of platinum bound to poly dG.poly dC is much larger than that of poly d (G.C) after incubation with equal quantities of reagent. Other workers, utilizing techniques to quantitate the bound platinum, have confirmed the dependence of covalent DNA-

platinum (II) binding on G.C content **[85]**. Platinum (II)-DNA intercalative binding is also a function of the G.C content of the biopolymer **[86]**.

Variations occur in platinum (II)-DNA binding with varying ionic strength or incubation times. At low (~1 mM) salt and high Pt-DNA phosphate ratios (r_f), $[Pt(en)Cl_2]$ and *E. coli* DNA show no further reaction after 12 hr at 20^0C **[87]**. In contrast, at r_f of 0.8 in 0.2 M NaCl and ambient temperatures with calf thymus DNA, differences were observed in the binding of $[(en)PtCl_2]$ for reaction times of 66 and 139 hr **[82(b)]**. Generally, low concentrations of a potentially competing ligand such as Cl^-, low total ionic strength, and long incubation times produced greater covalent platinum binding, provided that conditions are such as to prevent precipitation.

Although cis-$[Pt(NH_3)_2Cl_2]$ and $[Pt(en)Cl_2]$ have at least a kinetic preference for the base guanine **[67]**, platinum (II) binds to all the DNA bases. It has been reported **[88]** that at saturation with $[PtCl_3DMSO]^-$ both homopolymers and natural DNAs bind 2platinums/adenine, 1platinum/cytosine, 2platinums/guanine, and 0.6-0.8 platinum/thymine. The platinum product is trans-$[PtCl_2(DMSO)L]$, owing to the large kinetic trans effect of DMSO, and the DNAs are denatured by the binding. The binding sites are assumed to be the same as those reported in Table 1.9 for nucleosides. After 3 days incubation at 37^0C in 5 mM phosphate, cis-$[Pt(NH_3)_2Cl_2]$ was found **[71]** either to bind primarily to guanine (r_f = 0.2) or guanine and adenine (r_f = 0.4), whereas, cis- and trans-

$[Pt(NH_3)_2(H_2O)_2]^{2+}$ bound to all bases (r_f = 0.2). The binding sites in this latter study were also assigned to be the ring nitrogens designated in Table 1.9. X-ray photoelectron spectroscopic data have been interpreted to indicate a guanine 0-6, N-7 chelate with cis-$[Pt(NH_3)_2Cl_2]$ **[79]**. This conclusion is not supported by other work, however; Refs. **[67]** contain a good review of this subject **[136]**.

(d) Proteins : Potential Binding Sites :

Every protein or polypeptide chain has at least as many potential metal binding sites as there are peptide linkages. The carboxylate (C-terminal) and amino (N-terminal) ends of the chain are also potential ligands. At neutral pH, both terminal groups are charged and interact electrostatically with any charged platinum species present. Both carboxylates and amines can bind platinum (II). Carboxylates have fairly low nucleophilic reactivity constants, however, and are reasonably good leaving groups. Conversely, the affinity of amines for platinum (II) is high and these ligands are known to be very poor leaving groups, unless trans-labilized. The N-terminus is thus a better ligand for divalent platinum than the C-terminus. The amide nitrogens and the carbonyl oxygen atoms of the peptide linkages have a low affinity for platinum (II). These residues are present in much larger concentrations than any of the individual side chains in the majority of polymers. At least some of these amide groups are accessible on any protein or polypeptide, regardless of the molecule's

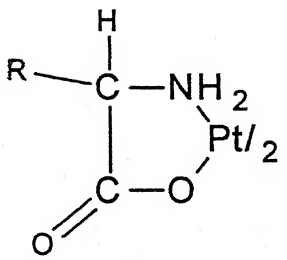
tertiary structure. Binding to peptide nitrogen and oxygen atoms, especially if deprotonated, should, therefore, be considered as a possibility.

Selected amino acids containing potential donors for platinum (II) on their side chains and corresponding affinities for the metal at neutral pH are summarized in Table 1.10. The sulfur-containing species cysteine and methionine have the highest affinity for platinum (II). Nucleophilicities of sulfur atoms are high (n^{o}_{Pt} values increase as RSH < R_2S < RS^-) and the Pt-S bond is kinetically inert. The pyridine-type imidazole nitrogen atom of histidine also has a high nucleophilicity for platinum (II) and is a poor leaving group. Arginine, although positively charged, is similar to imidazole, having one tertiary nitrogen atom that would have a reasonably high affinity for the metal. The primary amine lysine is positively charged at neutral pH and will bind to platinum (II) with loss of a hydrogen ion. The cyclic nitrogen atom of tryptophan is non-aromatic, but binding to platinum would be enhanced by loss of the proton. The remaining potential donors consist of carboxylates, amides, and alcohols, all of which have low or very low affinities for platinum (II) and are good leaving groups. Platinum (II) is thus, expected to bind to sulfur-containing residues and to imidazole rings whenever the tertiary structure of a protein or polypeptide renders these moieties available. Another possible binding site in proteins is the disulfide linkages formed by two cysteine residues. Platinum (II) will

bind to disulfides almost as readily as to Ocysteine or methionine.

(e) Platinum (II)-Amino Acid Binding :

The binding of platinum (II) to amino acids generally, and to sulfur containing units in particular, was reviewed in 1972 **[89,90]**. More current information is included in a recent review on platinum-protein binding **[91]**. Amino acids with no potential donor atoms in their side chains, e.g. glycine and alanine, bind platinum (II) through their terminal amino and carboxyl groups. At neutral pH stable, five-membered chelate rings form, whereas, under acidic or basic conditions the amino acid is monodentate. Under acidic conditions, the proton competes successfully with the metal for the carboxyl group and under basic conditions and sufficiently high amino acid concentrations the greater affinity of the amine group becomes evident.



There is some evidence based on spectroscopic assignments that the cis isomers of bis(amino acid) platinum (II) chelates are the kinetically preferred products at neutral pH when chloride is the leaving group **[89,91]**. It is suggested that a carboxylate oxygen atom binds the metal first, followed by ring closure with amine coordination, since both chloride

ions and amines are better trans labilizers than oxygen. Under basic conditions, however, where the amino group is expected to bind first, trans isomers of [Pt(L-proline)(L-alanine)] and [Pt(L-proline)(L-valine)] have been found **[92]**. The kinetic product, under basic conditions, in the reaction of $[Pt(DMSO)Cl_3]^-$ with glycine to form [Pt(DMSO)Cl(gly)] has the sulfur and nitrogen atoms trans to one another, whereas, the thermodynamically more stable product, obtained from heating the complex, is one in which oxygen is trans to sulfur **[93]**. In this latter complex, the donor atom with the lowest affinity for platinum (II) is trans to the donor with the highest affinity for the metal.

Dipeptides containing no potential ligands in their side chains bind to platinum (II) readily under basic conditions. Both peptide and terminal groups serve as ligands. Reaction of Zeise's salt, $[Pt(C_2H_4)Cl_3]^-$, with $(L\text{-valine})_2$, L-leucine-L-valine, and L-valine-L-leucine gave bridged binuclear products with the amino terminal nitrogen and peptide oxygen atoms of one amino acid bound to one cis-$[Pt(C_2H_4)Cl]^+$ entity and the peptide nitrogen and terminal carboxylate oxygen atoms of the second amino acid bound to another platinum (II) unit **[94]**.

Amino acids containing potential donor atoms in their side chains may bind to platinum (II) through these residues and/or through their terminal groups. The binding of platinum (II) to methionine occurs through the sulfur atom and with chelate ring formation through both terminal groups **[89-91]**. The reaction of any platinum (II) complex containing a labile

ligand with the methionine sulfur is rapid. The trans labilizing effect of the bound sulfur atom often aids the addition of a second methionine. For example, the reaction under basic conditions of cis-$[Pt(NH_3)_2Cl_2]$ with methionine yields $[Pt(NH_3)(met)_2]$, in which one methionine is bidentate and the other monodentate. The sulfur atoms are trans to one another. The reaction of trans–$[Pt(NH_3)_2Cl_2]$ with methionine, however, yields trans $[Pt(NH_3)(met)_2]$, in which both amino acids are monodentate. The latter reaction product is stable to all ligands except those having higher nucleophilicities than sulfur for platinum (II). Reaction of trans-$[Pt(NH_3)_2(met)_2]$ with iodide yields trans-$[Pt(NH_3)_2I_2]$ **[89-90]**. Under all conditions, the highest methionine-platinum (II) ratio observed is 2 : 1. Less work has been reported involving cysteine and platinum (II), although the metallointercalation reagent $[Pt(terpy)cys]^+$ has been synthesized **[82(b)]**. Cysteine coordinates through the sulfur atom, which loses a proton upon binding, but at pH $\geq$ 8 the complex decomposes, presumably with loss of terpyridine resulting from attack by the terminal amine of cysteine.

Histidine reacts very slowly with platinum (II) under basic conditions to form a bis-chelated complex **[89-91]**. Coordination is reported to be through the imidazole and the α-amino nitrogen atoms. Reactions of $[PtX_4]^{2-}$ (X = halide) or $[Pt(C_2O_4)_2]^{2-}$ with N-methyl imidazole (MeIm) yield mixtures of cis- and trans-$[Pt(MeIm)_2X_2]$, the cis compound exclusively, or $[Pt(MeIm)_4]^{2+}$ depending upon X^- and the conditions **[95,96]**. Trans-$[Pt(NH_3)_2(MeIm)_2]^{2+}$ is also known **[97]**.

Information about platinum binding to the other amino acids listed in Table 1.10 is scarce or non-existent. Platinum (II) binds to both L-lysine and L-aspartate **[98]**. No evidence for binding to tyrosine, tryptophan, serine, or threonine has been reported.

(f) Binding to Proteins and Polypeptides :

Many platinum (II)-protein binding sites have been determined by x-ray crystallography, since platinum (II) is one of several metals employed as a heavy atom label for proteins. An extensive review of the use of platinum (II) complexes to help phase protein crystal structures is available **[99]**. A less comprehensive but more encompassing review on complexes of platinum with proteins has also recently appeared **[91]**.

As with nucleic acids, the extent and type of platinum (II)-protein binding is a function of the composition of the platinum complex, the composition and tertiary structure of the protein, the ionic strength and composition of the medium, and the time allowed for incubation. Complexes such as $[Pt(CN)_4]^{2-}$ with no labile ligands bind proteins electrostatically, while complexes such as $[PtCl_4]^{2-}$ generally form covalent protein linkages. Charged complexes such as $[Pt(CN)_4]^{2-}$ and $[Pt(NH_3)_4]^{2+}$ are less likely to penetrate into the hydrophobic interior of proteins than are neutral species such as $[Pt(en)Cl_2]$.

The composition and tertiary structure of a protein or polypeptide dictate the number of available binding sites. The binding of several cis-amine platinum (II) nitrate complexes with poly(L-glutamate), poly(L-aspartate), and poly(L-lysine) at

pH 6.4 in the absence of halide ion has been investigated **[100]**. With the molar concentration of the platinum only 5% that of amino acid monomers, the metal binds bifuctionally to the carboxylate side chains of poly(L-glutamate), monofunctionally to the carboxylate side chains of poly(L-aspartate), and either bi- or monofunctionally to the amino side chains of poly(L-lysine). The leaving group was the nitrate ion. The difference in binding observed between the glutamate and aspartate polymers is attributed to the difference in length of the side chains of the two amino acids. Geometry prohibits bifunctional binding to nearest neighbours on the shorter aspartate side chains. Although the lysine residue is long enough to allow nearest-neighbour, bifunctional binding, the two possible binding modes could not be distinguished from each other. The polypeptides were all assumed to be in a random coil conformation.

The importance of the ionic strength and composition of the medium is illustrated by the following observations. The reaction between α-chymotrypsin crystals and $[PtCl_4]^{2-}$ is slow and non-reproducible in an ammonium sulfate medium. This reaction proceeds both more rapidly and with a higher degree of reproducibility in a phosphate medium **[101]**. The differences in reactivity are attributed to the formation of $[Pt(NH_3)_xCl_{4-x}]^{-2+x}$ species in the $(NH_4)_2SO_4$ medium. Ammonia is a very poor leaving group unless trans labilized. The $[PtCl_4]^{2-}$ ion does not bind to cysteine residues of triosephosphate isomerase in phosphate buffer, but does bind to these residues

within 2 days in ammonium sulphate **[102]**. Both solutions were at pH 7. Additionally, ribonuclease S binding to $[PtCl_4]^{2-}$ is limited to a methionine residue at pH 5.5 while at pH 7, binding includes a histidine.

The platinum (II) binding sites on various proteins as determined by x-ray crystallography are listed in Table 1.11. In each case the designated platinum reagent was added to a protein crystal. Both covalent and ionic binding sites are given. A large number of the covalent binding sites are methionines, especially when the reagent is K_2PtCl_4. Indeed, $[PtCl_4]^{2-}$ is considered to be methionine specific under appropriate reaction conditions and short incubation times **[103]**. It has also been proposed that the platinum, at least in some cases, is oxidized to platinum (IV) **[103]**. This proposal has met with criticism from a number of other workers, however, **[89,91,99]**. The ionic associations of $[Pt(CN)_4]^{2-}$ contrast quite sharply with the covalent binding sites of $[PtCl_4]^{2-}$, The former complex is more likely to bind charged amine residues than methionine, cysteine, or disulfide bonds, Identification of $[Pt(CN)_4]^{2-}$ binding sites, either by x-ray crystallography or nmr spectroscopy, is instructive in determining possible in vivo anion binding sites of proteins.

In recent years, various workers have studied the effect of platinum (II) complexes on enzymes such as leucine aminopeptidase and several dehydrogenases. The results of these experiments are summarized in a recent review **[91]**. The complexes $[PtX_4]^{2-}$ (X= halide ion), cis- and trans-$[Pt(NH_3)_2Cl_2]$,

and [Pt(en)Cl_2] have been studied most extensively and, although the enzyme binding sites have not been identified, the evidence points to sulfur-containing residues as the site of covalent interaction.

(g) Other Biological Molecules :

In addition to binding proteins and nucleic acids, platinum (II) has been shown to interact with smaller molecules of biological importance. The binding of platinum (II) to thiamine (vitamin B_1) and its phosphate esters has been reported **[104]**. The metal binds, not to the sulfur of the five-membered ring, but to the cyclic nitrogen of the six-membered ring, para to the exocyclic amino group. Platinum (II) porphyrins are also known. They are generally very stable and their solutions are strongly phosphorescent **[105]**.

1.5 : The Platinum Blues : A Case Study:

(a) Statement of the Problem :

Because many platinum (II) complexes are yellow or red, the occurrence of a blue product in the reaction of a platinum (II) salt has attracted particular attention. The first report of such a compound was issued at the turn of the century **[106]**, but it was not until quite recently that the molecular and electronic structures of any platinum blues were elucidated. The original *platinblau* was obtained in a reaction between [$(CH_3CN)_2PtCl_2$] and silver salts; it was formulated as a mononuclear platinum (II) acetamide complex, [$(CH_3CONH)_2Pt].H_2O$. The acetamide ligand arose through the

hydrolysis of acetonitrile during the course of the reaction. Unfortunately, the product was not crystalline, so the postulated structure could not be verified crystallographically after the subsequent advent of x-ray diffraction methods.

Various workers investigated or at least thought about the unusual blue platinum complexes in later years **[107-110]**. A blue, crystalline material was isolated from the reaction of trimethylacetamide with $[(CH_2CN)_2PtCl_2]$ and it appeared that the structure would finally be elucidated by x-ray crystallography **[108]**. The crystals proved to be a 7 : 2 : 1 mixture of three components, the first two of which were yellow, crystalline products isomorphous with the blue crystals. The blue component was an amorphous solid, however, formulated as a mononuclear platinum (IV) compound $[(t\text{-}C_4H_9CONH)_2PtCl_2]$ on the basis of extensive spectroscopic analyses. By analogy, the original *platinblau* was assigned the formula $[(CH_3CONH)_2Pt(OH)_2]$.

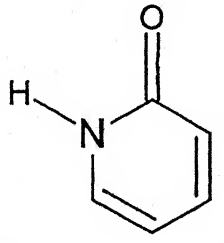
Blue platinum compounds also arise when divalent platinum complexes, such as the antitumor drug cis-$[PtCl_2(NH_3)_2]$ **[111]**, are allowed to react with pyrimidine bases or with DNA or RNA **[112]**. These bases, such as uracil and thymine (Fig.1.6), are cyclic amides that are presumed to serve the same ligand function as acetamide. Unfortunately, these blue compounds are also non-crystalline. Various investigations **[110,113-115]** revealed them to be paramagnetic oligomers of differing chain length, which

accounts for the difficulty in obtaining crystals. Platinum pyrimidine and amide blues have good antitumor drug activity with less nephrotoxicity than the parent cis-dichlorodiammineplatinum (II) **[116]**.

In order to achieve a full understanding of the platinum blues it was necessary to prepare a crystalline derivative, determine its structure, and compare its properties with other, non-crystalline members of the class. The fulfilment of this objective is described in the following sections together with the DNA-binding properties of platinum uracil blues.

(b) Molecular Structure of Platinum Blues :

A key to getting crystals of a blue platinum complex was to limit the oligomerization reaction. Since it seemed likely that the chain of platinum atoms is propagated through bridging amide ligands and hydrogen bonding interactions **[110]**, a ligand with minimal hydrogen bonding potential, α-pyridone, was employed. From a solution of hydrolysis products **[56]** of cis-diammineplatinum (II), α-pyridone, and sodium nitrate, kept at low pH and temperature to stabilize the blue colour,



dark blue parallelepipeds formed over a 12-hr period **[117,118]**. Analytical and x-ray crystallographic data revealed

the formula to be $[Pt_2(NH_3)_4(C_5H_4ON)_2](NO_3)_5.H_2O$ **[117,119]**. Two cis-diammineplatinum units are bridged by two deprotonated α-pyridone ligands.

The platinum atom at the chain end is bonded to two pyridine nitrogen atoms, while the inner platinum atom coordinates to the exocyclic oxygen atoms. The presence of these Pt-O bonds further stresses the point made earlier that; under the appropriate circumstances, the class 'b' platinum metal can coordinate strongly to oxygen. Two α-pyridonate bridged diplatinum units are further linked across a crystallographically required centre of symmetry. This linkage is supported by partial platinum-platinum bonding, discussed in the following section, and by four hydrogen bonds between the N-H protons of ammine ligands on one platinum atom and the acceptor oxygen atom on the adjacent platinum atom in the chain. The two centre platinum coordination planes are strictly parallel and eclipsed, whereas, the outer planes are canted by 27.4^0 with respect to one another and twisted by 22^0 about the Pt-Pt bond axis. The canting and twisting minimize non-bonded steric repulsions between the ammine ligands on adjacent planes and are features also observed in the closely related polymeric structure of cis-$[(NH_3)_4Pt_2P_2O_7]_n$ **[120]**.

The aromatic ring hydrogens of α-pyridone in the above structure preclude additional hydrogen-bonding interactions with adjacent tetranuclear cations and doubly bridged polymers do not form. Uracil and thymine have additional exocyclic oxygen atoms that could, and probably do, promote

polymerization. Various possible related structures for the platinum pyrimidine and acetamide blues have been discussed **[121,122]**.

(c) Electronic Structure of Platinum Blues :

Form the charge on the $[Pt_2(NH_3)_4(C_5H_4ON)_2]^{5+}$ cation it is apparent that platinum has a non-integral oxidation state of +2.25. Formally, then, the tetranuclear chain consists of three platinum (II) ions and one platinum (III) ion, accounting for the paramagnetism of the platinum blues. A temperature-dependent magnetic susceptibility study of cis-diammineplatinum α-pyridone blue showed it to be a simple Curie paramagnet with a magnetic moment of 1.81μ_B, consistent with the presence of one unpaired electron per tetranuclear unit **[119]**. The electron is delocalized over the four platinum atoms. Single-crystal electron spin resonance measurements revealed a nearly axial spectrum, with the unpaired spin residing in a molecular orbital comprised mainly of platinum d_{z^2} orbitals and directed along the chain axis. Although ^{195}pt hyperfine interactions were not observed in the solid state esr spectrum, they do appear in solution and reflect the delocalization of the unpaired electron along the platinum chain **[122]**. Similarly, an x-ray photoelectron spectral study of cis-diammineplatinum α-pyridone blue failed to detect any difference between the platinum-4f binding energies of the structurally distinguishable inner and outer pairs of platinum atoms **[118]**.

There are several lines of evidence that together establish that the molecular and electronic structures of cis-diammineplatinum α-pyridone blue are representative of the entire class of platinum blues. The solution optical, redox, and esr spectral properties of the compounds are very similar to one another **[113,119]**. The x-ray photoelectron spectra of the original *platinblau*, platinum uracil blue, and the α-pyridone blue are virtually identical **[118]**. Moreover, an EXAFS (extended x-ray absorption fine structure) investigation of cis-diammineplatinum uridine blue showed it to have a 2.9 Å Pt-Pt bond length and other features consistent with the structure of the α-pyridone blue **[123]**. It, therefore, appears that the platinum blues all share the features of bridging amidate ligands, mixed valency, and oligomerization typified by the tetranuclear cis-diammineplatinum α-pyridone blues.

The one remaining task is to define precisely the electronic transition responsible for the blue colour. It is clear that the band involves a transition either into or from the nearly filled molecular orbital housing the unpaired electron, since loss of colour and paramagnetism have been experimentally linked with one another **[122]**. Single-crystal optical studies would provide a definitive answer **[122]**.

(d) Solution and Redox Chemistry :

The colour of solutions of the platinum blues bleaches with time **[110,122]**. The absorbance of the blue chromophore depends on the anion present and is also sensitive to

temperature. Chloride ion discharges the blue colour, while addition of nitrate ion or lowering of the temperature stabilizes it. Kinetic studies reveal the decomposition of cis-diammineplatinum α-pyridone blue, followed by the disappearance with time of either the blue chromophore at 680 nm or the esr signal intensity, to be first-order in platinum at high concentration but higher-order at low concentration **[122].** This result indicates a multistep decomposition. For example, two tetramers may decompose to form a diamagnetic platinum (III) dimer, two platinum (II) dimers, and two platinum (II) monomers.

Oxidative titrations of cis-diammineplatinum α-pyridone blue with ceric ion show a linear decrease in esr signal intensity and A_{260} upon addition of three equivalents of oxidant **[122].** The product is presumably cis-$[Pt_2(NH_3)_4(C_5H_4NO)_2]^{4+}$, the platinum (III) dimer. A similar result was obtained by potentiometric titration, following which oxidation to platinum (IV) was observed **[124].** Reductive titrations with excess ferrous sulfate and back titration with permanganate give formal oxidation states of 2.27 ± 0.10 for cis-diammineplatinum α-pyridone blue, 2.08 ± 0.15 for platinum uracil blue, and 2.28 ± 0.17 for a green hypoxanthine analogue **[122].** These results and the similarity in optical and esr spectral properties of the platinum blues in solution further support the conclusion that they are mixed-valent oligomers. Gel electrophoretic studies of the cis-diammineplatinum blues

indicate the length of the oligomers to increase along the ligand series α-pyridone < hypoxanthine < uracil, assuming an identical charge per monomer unit **[122]**.

(e) Binding to DNA :

The DNA-binding properties of platinum uracil blue (PUB) have been extensively studied **[125]**. This compound has antitumor drug activity and has been used as a cytological stain **[126,127]**; both functions are likely to involve platinum-DNA interactions. At low salt concentration, 0.5 M or less, a precipitate forms between PUB and closed circular DNAs which redissolves by increasing the ionic strength to greater than 1.0 M. The buoyant density of the DNA in CsCl increases co-operatively with the concentration of PUB. Using [^{14}C]-radiolabeled uracil, it was shown that platinum binds to the DNA and releases its uracil ligand since no label is transferred to the DNA **[125]**. The reaction can largely be reversed with cyanide to form the very stable $[Pt(CN)_4]^{2-}$ ion. Nothing is known about the binding sites of platinum on the DNA, however, and future work will have to be directed at elucidating this feature.

1.6 : Detecting Platinum in Biological Systems :

The growing interest in studying the interaction of platinum (II) complexes with biological molecules has led to the development of analytical techniques for detecting platinum. While the effects of platinum of the properties (activity, electrophoretic mobility,

spectrum, etc.) of a biopolymer are readily monitored, it is usually desirable, upon separation of the biopolymer from a platinum reagent, to ascertain whether any platinum remains with the polymer and, if so, where and how much. A brief review of the analytical techniques employed to detect platinum in the presence of a biopolymer and to elucidate the nature of its binding are, therefore, provided in this section.

(a) Methods of Detection :

Platinum attached to a biopolymer can be determined either directly or indirectly. Indirect methods usually involve monitoring a radioactively labeled, kinetically inert ligand, or studying platinum loss in a solution from which the biopolymer has been removed. Direct determinations are preferable and will be stressed here. Unfortunately, such methods are not always feasible.

The direct detection of platinum can be accomplished by several methods. Perhaps the least expensive and most versatile of these is flameless atomic absorption spectroscopy. Platinum can be detected by this method, even in the presence of a biopolymer, in amounts as low as 1-20 μg when other metals are absent. Atomic absorption has been employed by a variety of workers for studying platinum bound to DNA **[79,80,128]**. There is no indication that the DNA, buffer systems, or composition of the platinum reagent employed interfere with platinum detection by this method.

Another method for directly determining platinum, applicable over a wide concentration range, is the use of the

radioactive isotope, ^{195m}Pt. This metastable isotope was used **[85]** to determine the quantity of cis-$[Pt(NH_3)_2Cl_2]$ bound to DNA. However, its short half-life (4.1 days) and limited availability limit the utility of this approach.

Platinum-195, the stable isotope, is 33.8% abundant and has a nuclear spin, I, of one-half. Detection of this species is, therefore, possible by nmr spectroscopy. ^{195}Pt nmr has not, as yet, been employed as a means of detecting platinum in biological system. The conditions for detection are concentrations in the millimolar range and, even with a ^{195}Pt-enriched sample **[129]**, many systems are too dilute for this method to become practical. ^{13}C and ^{1}H-nmr spectroscopy have been used to determine platinum binding to both nucleosides **[69,71,130]** and amino acids **[131]**. Platinum-195 coupling occurs with both ^{13}C and ^{1}H nuclei and in solutions of ~1 mM platinum concentration, this coupling can be readily observed at natural abundance. Concentration is again the limiting factor in applying this technique for platinum detection to biopolymers, however, Enrichment of platinum reagents with ^{195}Pt would lower the concentration requirements, but only by a factor of 3.

The presence of platinum in a biopolymer may also be determined by x-ray diffraction. The high electron density of platinum facilitates its identification in the presence of the lighter elements of which biopolymers are normally composed. Detection of platinum by this method is easiest when the

system is highly ordered. Platinum binding to proteins **[99]** and to tRNA **[75,76]** has been elucidated by x-ray crystallography and, in the case of the metallointercalation reagents, platinum binding to DNA has been ascertained by fiber x-ray diffraction analysis **[83(a)]**.

Other methods of directly detecting platinum are also available. Instrumental technique such as EXAFS and mass spectrometry have been used to measure distances between platinum and its ligands on DNA **[132]** and binding to nucleosides, respectively **[133]**. Colorimetric tests may also be used. Here the platinum-biopolymer complex is degraded, the platinum reduced to Pt (0), removed from any potential ligands, and then reoxidized to Pt (II) or Pt (IV). One such colorimetric test used p-nitrosodimethylaniline as the reagent and is optimal over a range of 3.6×10^{-6} to 1.2×10^{-5} M in platinum **[134]**.

Finally, the presence of platinum in a biopolymer may generate a new spectral band characteristic of a platinum-ligand bond. The presence of platinum on poly- and mononucleotides containg phosphorothioate groups was monitored **[78]** by the appearance of an electronic absorption band specific to sulfur-bonded platinum terpyridine systems. Platinum binding to polypeptides was ascertained **[100]** by charge transfer bands indicative of carboxylates or amines bound to platinum bipyridine and platinum o-phenanthroline systems.

(b) Nature and Amount of Bound Platinum :

Once platinum has been detected in a biochemical system, it is often of interest to learn where and how much of the meterial is bound. Covalent binding can be differentiated from electrostatic or other associative interactions by electrophoresis or sometimes by exhaustive dialysis of the platinum-biopolymer complex. Methods for determining intercalative binding to both linear and circular DNAs and for diffenciating between this type of binding and either covalent or electrostatic interactions have been thoroughly described **[82(b)]**. Here ethidium bromide fluorescence inhibition studies and sedimentation and electrophoretic studies on closed circular DNAs were employed.

The question of where the platinum is bound on the biopolymer is not always easy to answer and has been the topic of discussion in Sec. 1.4. How much of the platinum is bound can often be determined through the direct detection of platinum or through the various indirect methods mentioned above. Atomic absorption spectroscopy and radioactive isotopic labeling are two techniques that are excellent for quantitating the amount of bound platinum, but they give no information about where or how the metal is bound. It is rare that these techniques are employed merely to detect platinum without quantitation; the same is true of colorimetric analyses.

Conversely, nmr studies yield better information about where the platinum is bound than about how much. This

result is also true of Raman spectroscopy **[67,71,130]**, EXAFS **[132]**, x-ray photoelectron spectroscopy [79], and mass spectrometry **[132]**.

X-ray crystallography provides definitive information about where and how much platinum is bound. This technique is limited, however, to fairly small nucleic acids or oligonucleotides and to proteins. Fiber diffraction analyses of larger, polycrystalline nucleic acids can be instrumental in determining where the platinum is, but reveal little about how much metal is bound.

Systems where platinum binding gives rise to a spectroscopic change, such as charge transfer bands in the ultraviolet or visible portion of the spectrum, are generally excellent for determining both where and how much of the metal is bound **[78]**.

Table 1.1 :

Overall Formation Constants for Platinum (II) Complexes in Water at 25°C[a]

Ligand	Complex	log β_4
CN^-	$[Pt(CN)_4]^{2-}$	41
NH_3	$[Pt(NH_3)_4]^{2+}$	35.3
OH^-	$[Pt(OH)_4]^{2-}$	35
I^-	$[PtI_4]^{2-}$	29.6
Br^-	$[PtBr_4]^{2-}$	20.5
Cl^-	$[PtCl_4]^{2-}$	16.6

[a]Equilibrium constants for the reaction $Pt^{2+} + 4L^{-x} \rightleftharpoons PtL_4^{(2-4x)}$ are taken from Ref.**[10]** .

Table 1.2 :

Equilibrium Constants, K, for Some Platinum (II) Substitution Reactions in Water at 25°C[a]

$[Pt(C_2H_4)Cl_3]^- + X^- \rightleftharpoons$ trans- $[Pt(C_2H_4)Cl_2X]^- + Cl^-$							
X^-	NH_3	OH^-	SCN^-	I^-	Br^-	F^-	H_2O
log K	7.8	6	>2	2	0.5	-1.5	-2.5
trans- $[Pt(NH_3)_2(OH)_2] + 2X^- \rightleftharpoons$ trans- $[Pt(NH_3)_2X_2] + 2OH^-$							
X^-	I^-	SCN^-	Br^-	Cl^-			
log K	-6.3	-6.6	-8.6	-10			
$[Pt\{(CH_3)_2S\}Cl_3]^- + CH_3OH \rightleftharpoons$ trans-$[Pt\{(CH_3)_2S\}(CH_3OH)Cl_2]^+ + Cl^-$							
log K	-2.85[b]						

[a]Data have been taken from Ref. **[10]**, except where noted.

[b]Data from Ref. **[17]** at 30°C in methanol.

Table 1.3 :

Selected Platinum (II) Reduction Potentials[a]

Half-reaction	E^0 (v)
$Pt^{2+} + 2e^- \rightleftharpoons Pt$	1.2
$Pt(OH)_2 + 2e^- \rightleftharpoons Pt + 2OH^-$	0.14
$PtCl_4^{2-} + 2e^- \rightleftharpoons Pt + 4Cl^-$	0.75
$PtBr_4^{2-} + 2e^- \rightleftharpoons Pt + 4Br^-$	0.67
$PtI_4^{2-} + 2e^- \rightleftharpoons Pt + 4I^-$	0.40
$PtCl_6^{2-} + 2e^- \rightleftharpoons PtCl_4^{2-} + 2Cl^-$	0.77
$PtBr_6^{2-} + 2e^- \rightleftharpoons PtBr_4^{2-} + 2Br^-$	0.64
$PtI_6^{2-} + 2e^- \rightleftharpoons PtI_4^{2-} + 2I^-$	0.39

[a]Data have been taken from Ref. **[1]**.

Table 1.4 :

Examples of Oxidative Addition Reactions of Pt (II) Complexes in Aqueous Media

Reaction	Ref.
$[Pt(CN)_4]^{2-} + I_2 \rightleftharpoons$ trans- $[Pt(CN)_4I_2]^{2-}$	18
$[Pt(NH_3)_4]^{2+} + H_2O_2 \rightleftharpoons [Pt(NH_3)_4(OH)_2]^{2+}$	19
$2[Pt(NH_3)_4]^{2+} + 2S_2O_8^{2-} + H_2O \rightleftharpoons [Pt(NH_3)_4(OH)(SO_4)]^{+}$ + $[Pt(NH_3)_4(SO_4)_2] + SO_4^{2-} + H^{+}$	19
trans-$[Pt(NH_3)_2Cl_2] + Cl_2 \rightleftharpoons$ trans-$[Pt(NH_3)_2Cl_4]$	20

Table 1.5 :

Nucleophilic Reactivity Constants, n^0_{Pt} values[a]

Nucleophile	n^0_{Pt}	Nucleophile	n^0_{pt}
CH_3OH	0.00	C_6H_5SH	4.15
CH_3COO^-	<2.0	Br^-	4.18
F^-	<2.2	$S(4\text{-}NH_2C_6H_4)_2$	4.27
α-Picoline	2.2	$S(C_2H_5)_2$	4.52
CH_3O^-	<2.4	$P[N(C_2H_5)_2]_3$	4.54
$S(4\text{-}NO_2C_6H_4)_2$	2.4	$S(CH_3)_2$	4.87
Cl^-	3.04	$PO(OCH_3)_2^-$	5.01
NH_3	3.07	$S(CH_2)_5$	5.02
Piperidine	3.13	$S(CH_2)_4$	5.14
Aniline	3.16	I^-	5.46
Pyridine	3.19	$Se(CH_2C_6H_5)_2$	5.53
$S(4\text{-}ClC_6H_4)_2$	3.21	$Se(CH_3)_2$	5.70
NO_2^-	3.22	SCN^-	5.75
$S(C_6H_5)_2$	3.22	SO_3^{2-}	5.79
$S(C_6H_5)$ $(4\text{-}ClC_6H_4)$	3.25	$C_6H_{11}NC$	6.34
$S(4\text{-}FC_6H_4)_2$	3.30	$Sb(C_6H_5)_3$	6.79
$S(4\text{-}NH_2C_6H_4)$ $(4\text{-}NO_2C_6H_4)$	3.31	$As(C_6H_5)_3$	6.89
$S(CH_2C_6H_5)_2$	3.43	$SeCN^-$	7.11
Imidazole	3.44	CN^-	7.14
N_3^-	3.58	$C_6H_5S^-$	7.17
$S(C_6H_5)(4\text{-}CH_3OC_6H_4)$	3.64	$SC(NH_2)_2$	7.17
$S(4\text{-}CH_3C_6H_4)_2$	3.68	$P(OCH_3)_3$	7.23
$S(4\text{-}CH_3OC_6H_4)_2$	3.73	$S_2O_3^{2-}$	7.34
NH_2OH	3.85	$As(C_2H_5)_3$	7.68
$S(4\text{-}OHC_6H_4)_2$	3.85	$P(C_6H_5)_3$	8.93
NH_2NH_2	3.86	$P(C_2H_5)_3$	8.99

[a]Data have been taken from Refs. **[1]** and **[34]**.

Table 1.6 :

Equilibrium Constants for Hydrolysis Reactions[a]

(Reactant + H_2O $\rightleftharpoons$ product + X)

X	Product	K_{eq} (mM^{-1})	T(°C)	Ref
Cl^-	$[(en)Pt(H_2O)Cl]^+$	2.19	25	**57**
Cl^-	$[(en)Pt(H_2O)Cl]^+$	2.76	35	**57**
Cl^-	$[(en)Pt(H_2O)_2]^{2+}$	0.143	25	**57**
Cl^-	$[(en)Pt(H_2O)_2]^{2+}$	0.138	35	**57**
Cl^-	cis-$[(NH_3)_2Pt(H_2O)Cl]^+$	3.63	25	**47(a)**
Cl^-	cis-$[(NH_3)_2Pt(H_2O)Cl]^+$	4.37	35	**47(a)**
Cl^-	cis-$[(NH_3)_2Pt(H_2O)_2]^{2+}$	0.111	25	**47(a)**
Cl^-	cis-$[(NH_3)_2Pt(H_2O)_2]^{2+}$	1.88	35	**47(a)**
Br^-	cis-$[(NH_3)_2Pt(H_2O)Br]^+$	1.13	25	**47(b)**
Br^-	cis-$[(NH_3)_2Pt(H_2O)_2]^{2+}$	0.042	25	**47(b)**
Cl^-	trans-$[(NH_3)PtCl_2(H_2O)]$	14	25	**28**
Cl^-	cis-$[(NH_3)_2PtCl_2(H_2O)]$	<0.25	25	**28**
Cl^-	$[PtCl_3(H_2O)]^-$	12.6	25	**58**
Cl^-	$[PtCl_3(H_2O)]^-$	8.0	25	**16**
Cl^-	$[Pt(Cl)(H_2O)_3]^+$	0.10	25	**16**
Cl^-	$[Pt(H_2O)_4]^{2+}$	0.011	25	**16**
Cl^-	trans-$[PtCl_2(H_2O)_2]$	1.2	25	**28**
Cl^-	cis-$[PtCl_2(H_2O)_2]$	0.80	25	**28**
Cl^-	trans-$[(DMSO)PtCl_2(H_2O)]$	0.013	25	**59**
Cl^-	trans-$[(DMSO)PtCl_2(H_2O)]$	5.2	25	**28**
Cl^-	cis-$[(DMSO)PtCl_2(H_2O)]$	0.115	25	**28**
Cl^-	cis-$[(C_2H_4)PtCl_2(H_2O)]$	<0.25	25	**28**
Cl^-	trans-$[(C_2H_4)PtCl_2(H_2O)]$	3	25	**28**

[a]K_{eq} values correspond to the monohydrolysis reaction.

Table 1.7 :

Rate Constants for Hydrolysis Reaction, k, and for the Reversible Halide Anations, K^{-1}, at 25°C

Leaving Group	Product	k(sec^{-1})	K^{-1} ($sec^{-1}M^{-1}$)	Ref.
Cl^-	$[(en)PtCl(H_2O)]^+$	3.4×10^{-5}	1.5×10^{-2}	**57**
Cl^-	$[(en)Pt(H_2O)_2]^{2+}$	4.4×10^{-5}	3.1×10^{-1}	**57**
Cl^-	$cis\text{-}[(NH_3)_2PtCl(H_2O)]^+$	2.5×10^{-5}		**8**
Cl^-	$cis\text{-}[(NH_3)_2Pt(H_2O)_2]^{2+}$	3.3×10^{-5}		**8**
Cl^-	$trans\text{-}[(NH_3)_2Pt(H_2O)Cl]^+$	9.8×10^{-5}	3×10^{-4}	**8**
Cl^-	$trans\text{-}[(NH_3)PtCl_2(H_2O)]$	4×10^{-6}	2.7×10^{-4}	**8**
Cl^-	$cis\text{-}[(NH_3)PtCl_2(H_2O)]$	3×10^{-5}	2.3×10^{-5}	**8**
Cl^-	$cis\text{-}[(NH_3)PtCl(H_2O)_2]^+$	5.9×10^{-5}[a]	9×10^{-5}	**8**
Cl^-	$trans\text{-}[Pt(H_2O)_2Cl_2]$	2.8×10^{-8}	3.8×10^{-2}	**28**
Br^-	$trans\text{-}[Pt(H_2O)_2Br_2]$	1.4×10^{-8}	18.2	**28**
Cl^-	$cis\text{-}[Pt(H_2O)_2Cl_2]$	3×10^{-5}	54	**28**
Cl^-	$trans\text{-}[(DMSO)PtCl_2(H_2O)]$	0.10	1.7	**28**
Br^-	$trans\text{-}[(DMSO)PtBr_2(H_2O)]$	0.10	390	**28**
Cl^-	$cis\text{-}[(DMSO)PtCl_2(H_2O)]$	2×10^{-4}	6000	**28**
Cl^-	$cis\text{-}[(DMSO)PtCl(H_2O)_2]^+$	0.34[b]	3900	**28**
Br^-	$cis\text{-}[(DMSO)PtBr(H_2O)_2]^+$	1.5[b]	0.7	**28**
Cl^-	$[(DMSO)Pt(H_2O)_3]^{2+}$	0.47[b]		**28**
Cl^-	$[(DMSO)Pt(H_2O)_3]^{2+}$	1.3×10^{-6}[c]		**28**

[a]Leaving group is trans to NH_3.

[b]Leaving group is trans to DMSO.

[c]Leaving group is cis to DMSO.

Table 1.8 :

pK_a Values of Platinum(II) Hydrolysis Products

Complex	pK_a	T(°C)	Ref.
$[Pt(en)(H_2O)_2]^{2+}$	5.8	25	**56**
$[Pt(en)(H_2O)(OH)]^{+}$	7.6	25	**56**
cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$	5.6	20	**56**
cis-$[Pt(NH_3)_2(H_2O)(OH)]^{+}$	7.3	20	**56**
$[Pt(dien)(H_2O)_2]^{2+}$	6.13	25	**61**
$[Pt(H_2O)_4]^{2+}$	>2.5	25	**62**
$[PtCl_3(H_2O)]^{-}$	~7	25	**63**

Table 1.9 :

Known Platinum(II) Binding Sites on Nucleic Acid Constituents in the pH Range 6-8.5[a]

Complex	Coordination site	Type of evidence
$[Pt(adenosine)_4]^{2+}$	N-7	^1H nmr
cis-$[Pt(NH_3)_2(adenosine)_2]^{2+}$	N-7 and / or N-1	Ultraviolet spectroscopy; pH titrations
$[Pt\{(dien)\}_2(adenosine)]^{4+}$	N-7 and N-1	^1H nmr
$[Pt(en)(guanosine)_2]^{2+}$	N-7	X-ray crystallography
cis-$[Pt(NH_3)_2(guanosine)_2]^{2+}$	N-7	X-ray crystallography
trans-$[\{Pt(NH_3)_2(OH)\}_2(5'\text{-GMP})]$	N-7 and N-1	Raman spectroscopy[b]
$[Pt(en)(5'\text{-CMP})]_2.\ 2H_2O$	N-3 and O (phos)	X-ray crystallography
$[Pt(dien)(uridinate)]^+$	N-3	^1H nmr[c]
$[Pt(dien)(thymidinate)]^+$	N-3	^1H nmr[c]
$[Pt(en)(uridinate)_2]$	N-3	^1Hnmr[c]

[a]Data have been taken from Refs. **[67]** and **[68]**.

[b]Data have been taken from Ref. **[69]**.

[c]Data have been taken from Ref. **[70]**.

Table 1.10 :

Amino Acids Containing Donor Atoms for Platinum(II)[a]

Amino acid	**pK_a**	**Residue serving as binding site**	**Affinity for Pt(II)**
Cysteine	8.3	Sulfur atom	Very high
Methionine		Sulfur atom	Very high
Histidine	6.00	Imidazole nitrogen	High
Arginine	12.48	Amine	Moderate
Lysine	10.53	Amine	Moderate-low
Tryptophan		Cyclic nitrogen atom	Moderate-low
Aspartic acid	3.86	Carboxylate	Low
Glutamic acid	4.25	Carboxylate	Low
Asparagine		Amide	Low
Glutamine		Amide	Low
Tyrosine	10.07	Phenolate	Low
Serine		Alcohol	Very low
Threonine		Alcohol	Very low

[a]Amino acids are listed in approximate order of affinity for platinum(II) at neutral pH.

Table 1.11: Platinum (II) Binding Sites on various Proteins[a]

Protein	Reagent	Reagent Concentration (mM)	Buffer[b]	pH	Time of soak	Site No.	Z	Binding site
Concanavalin A	K_2PtCl_4	0.5	2.1 M phosphate	6	3 days	1	61	Met 129,His 127
						2	23	Met 129
Chironomus hemoglobin	K_2PtCl_4		3.75 M phosphate	7		1	80	Met H 17
						2	55	His G2, C-terminus
						3	7	His G19
Chironomus hemoglobin	$Pt(NO_2)_2(NH_3)_2$		3.75 M phosphate	7		1	45	His G2
						2	74	His G19
Ribonuclease S	$Pt(en)Cl_2$	2	3.2 M AS	8		1		His 119
Ribonuclease S	$Pt(en)Cl_2$	2	3.2 M AS	5.5	30-50 hr (fresh sol,n every 10 hr)	1	64	Met 29
Lactate dehydrogenase	$Pt(en)Cl_2$	2.5				1	32	Cys (SH)
						2	81	Cys (SH)
Concanavalin	K_2PtCl_4	1			2 days	1	75	His 127, Met 129
						2	11	Met 42
Horse ferrocyto-chrome c	K_2PtCl_4		4.6 M phosphate	6.2		1	35	Met 65
						2	40	Met 65
						3	8	His 33
Tuna Ferricytochrome c	K_2PtCl_4	0.1	95% AS	6	2 days	1	24	Met 65
Cytochrome c_{550}	K_2PtCl_4	1.3			7 days	1	69	
α-Chymotrypsin	K_2PtBr_4, K_2PtCl_4or K_2PtI_4		3.5 M phosphate 2-4% dioxane	4.2		1,2	95	N-terminus and S-S of Cys 1-127
						3,4	55	Met 192

Table 1.11: Continued

Protein	Reagent	Reagent Concentration (mM)	Buffer[b]	pH	Time of soak	Site No.	Z	Binding site
Subtilisin BPN`	K_2PtCl_4	0.65	2.1 M AS 00.05 M acetate	5.9	10-40 days	1 2	78 14	Met 50 His 64
Subtilisin novo	K_2PtCl_4					1 2		Met 50 Trp 241, His2 Trp 106
						3		Ala1(N-ermin
Thermolysin	K_2PtCl_4	6	5% DMSO 0.01$CaAc_2$ 0.01 M tris/ acetate	5.8	10 days	1 2		His 250 His 126
Carboxypeptidase A	K_2PtCl_4		0.2 M LiCl 0.02 M tris	7.5	42 days	1 2 3 4	74 45 68 27	Cys 161 (-S-S) Met 103 N-terminus:Ala His 303
High potential Iron protein (HiPIP)	$K_2Pt(NO_2)_4$	10	3.2 M AS	6.5	7 days	1 2		Met 49 (major site)
Adenyl kinase	$K_2Pt(NO_2)_4$	2			68 days	1 2	40 17	His 36 (major site)
Carbonic anhydrase	MMTGA + $K_2Pt(CN)_4$		2.3 M AS	8.5		1		Zn,Thr197, X139
Ribonuclease S	$K_2Pt(CN)_4$	5	3 M AS 0.1 M acetate	5.5		1 2 3 4 5	24 28 16 12 8	

Tab;e 1.11: Continued

Protein	Reagent	Reagent Concentration (mM)	Buffer[b]	pH	Time of soak	Site No.	Z		Binding site
Tuna ferrocytochrome c	$K_2Pt(CN)_4$	6	95% AS	6	1 day	1	30		Nonear neighbor, Lys 53, Ala 4, Lys 7
							2	15	Ser 100, Val 3, Glu 44, Gln 70, Lys 72, Lys 73
							3	9	Lys 99, Lys 99`, Ser 103.
							4	5	Ser 103`, Glu 21, Lys 7, Lys 25
							5	6	Ile269(mainchain)
Liver alcohol dhydrogenase	$K_2Pt(CN)_4$	1	0.05 M tris/HCL	8.4	Cocryst	1			Asp 223, Lys 228, Arg 47, Arg 369
Adenyl kinase	$K_2Pt(SCN)_4$	2			8 days	1	90		
							2	38	Major site near
							3	27	His 36
							4	19	

[a]Data are taken from Ref. [99]

[b]AS = ammonium sulphate

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CHAPTER-II

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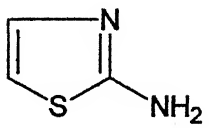
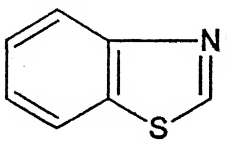
2.1: INTRODUCTION

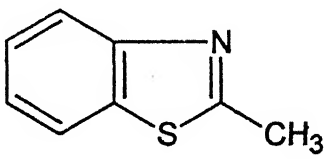
Complexes of divalent platinum are very rare. A survey of the literature of neutral mixed ligands complexes of Pt (II) reveals few reports on such compounds. Although considerable importance is involve in the study of mixed ligand Platinum complexes having d^8 electronic configuration with 2-amino thiazole, benzothiazole and 2–methyl benzothiazole have been rarely described. Further, there is no report on mixed ligand divalent platinum complexes with benzothiazoles and thiazole derivatives.



2-Aminothiazole



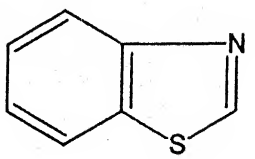
Benzothiazole



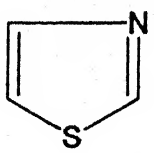
2–Methyl benzothiazole

As this chapter involves the synthesis of complexes with benzothiazoles based organic compounds, it would be appropriate to discuss here the structure, vibrational spectra, UV-Vis spectra and NMR spectral features of benzothiazoles and its derivatives.

Benzothiazole *(structure I)* and its derivatives are basically having bicyclic fused ring systems where one benzene ring is fused to the 4,5 positions of the thiazole ring *(structure II)*



structure I



structure II

Benzothiazole and its derivatives are particularly important because of their commercial and biological interest developed due to *'Thiazole system'*.

In 1935, Williams and co-workers demonstrated the existence of the simple thiazole ring in vitamin B_1 (Thiamine) **[1]**. Shortly thereafter, the development of sulfa drugs led to recognition of the usefulness of sulphathiazole and several its derivatives as chemotherapeutic agents for the treatment of bacterial infections **[2]**. The biological importance of thiazole derivatives was further emphasized during the period 1941-45 when work on the structure of the antibiotic, pencillin, showed the presence of a thiazolidine ring in this important therapeutic agent **[3]**.

2.1.1 : Structure of Thiazole :

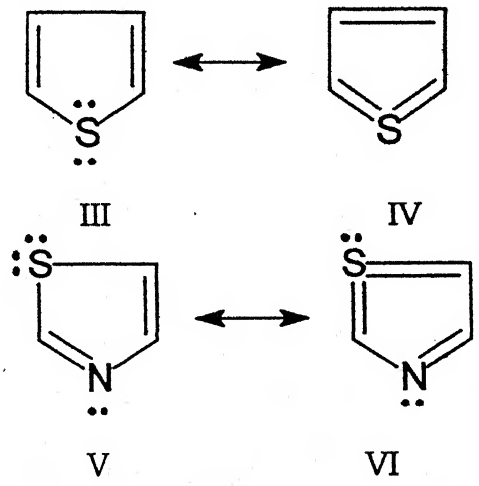
In general, thiazole ring system exhibits marked aromatic character **[4]**, and is structurally related to thiophene and pyridine but many of its chemical, physical **[5]** and biological properties **[6]** are close to the latter. Thiazole is a colourless liquid, b.p. 117°C which is close to pyridine (115°C) and thiophene (84°C). It resembles pyridine in odour and is miscible in water. These striking similarities between the thiazoles and pyridine led to an early recognition that the substitution of the sulfur atom for a vinylene group (-CH=CH-) in a conjugated ring system results in the substances of similar properties. The classical structural representation (II) of the thiazole ring system places the double bond in the 2,3 and 4,5 positions only. This fixation of double bonds does not express adequately the 'aromatic nature' of the thiazole ring.

Bamberger **[7]** attempted to represent the aromatic character by considering the sulfur atom in these cyclic systems to be tetravalent. This permits to express the similar nature of these five and six membered 'aromatic sextet' of electrons.

In the early interpretation of such structures, each centric bond was considered to represent one electron to give the 'aromatic sextet' of electrons. In the six membered rings, each ring atom contributes one electron to the sextet, but in the sulfur containing five membered rings the sulfur is required to contribute two electrons.

With the development of the electronic concept of atomic structure and the application of the octet rule led to a reconsideration of the classical structure of the thiazole. Hans Erlenmeyer and collaborators **[8]** extended the concept of isosterism to thiazole and thiophene series. In this concept, the vinylene (-CH=CH-) group of an aromatic ring and the sulfur atom of the thiophene and thiazole ring are considered isosteric by virtue of each group's having the same number of binding electrons **[8b,d]**. Thus, the group $-\ddot{C}=\ddot{C}-$ (H H) and the sulfur atom, $-\ddot{S}-$, become electronically similar and vinylene group is considered as a 'pseudo sulfur atom **[8b,d]**. Although this broadened concept of isosterism as put forth by Erlenmeyer is helpful with thiophene and thiazole, it is inadequate when applied to the oxygen and nitrogen isosteres, furan pyrrole, oxazole, and isoxazole **[9]**.

The inadequacy of isosterism led to the application of the concept of resonance to the structure of both thiophene and thiazole **[9,10]**. Within the last few years, studies of the absorption spectra **[11]**, dipole moments **[12,13]** and electron diffraction patterns **[13]** of a variety of sulfur containing compounds have shown that a sulfur atom does not always obey the octet rule and can accommodate a decet of electrons in its outer valence shell. These findings allow the consideration of many more electronic structures for the sulfur containing heterocycles and lead to the equivalence of not only –H=CH– and –S̈– but also of =CH–CH= and =S̈= wherein, the sulfur has an expanded outer shell. From these concepts, Erlenmeyer has advanced analogous structures (V,VI) for the thiazole ring and has presented some chemical evidence in support of a significant contribution by (VI).



The electronic structure of thiazole has been approached using various theoretical methods. The best set of numerical values introduced into simple Huckel type MO calculation is,

for Coulomb integrals, $\alpha_N = \alpha + 0.5\beta$, $\alpha_S = \alpha_C$, and for resonance integrals, $\beta_{CN} = \beta_{CS} = \beta_{CC} = 0.5$. The resulting net π charges, π bond orders and free valence electrons are given in Figure 2.1 and the calculated localization energies are given in Table 2.1. More elaborate treatments have also been applied to thiazole including *ab initio* methods **[14,15]** and all valence electron methods **[16,17]** (PPP and CNDO). Figure 2.2 reproduces the total net charge ($\sigma+\pi$) given by an *ab initio* calculation. Whatever, the methods employed, common trends can be noted in the electronic properties of the thiazole molecule. In all the cases the net π-charge on sulfur is positive, whereas, its net σ-charge is sometimes is positive, sometimes negative. The all electrons *ab initio* method gives a positive total net charge. In all the cases, the total net charge on nitrogen is negative. Of the three carbon atoms of the ring situation is clear for C-2 only; its total net charge is either positive or very close to zero, while its net π-charge is positive. The charges at C-4 and C-5 are not uniform; the net π-charge at C-4 is very low, whereas, that at C-5 is slightly negative. From their net σ-charges the three hydrogen atoms are predicted to have decreasing acidity in the order H-2 $\geq$ H-5 > H-4, which is consistent with experimental results. The distribution of the π-bond orders gives a picture of the distribution of π-electrons along the σ-frame of the ring and, therefore, of its aromaticity which can be confirmed by NMR and UV spectroscopic methods. Thiazole is slightly aromatic with a certain aromatic character. The two bonds to the nitrogen are different; N-C(2), having the more π-

character than N-C(4), should be shorter, which is confirmed by microwave spectroscopy. The free valencies calculated by

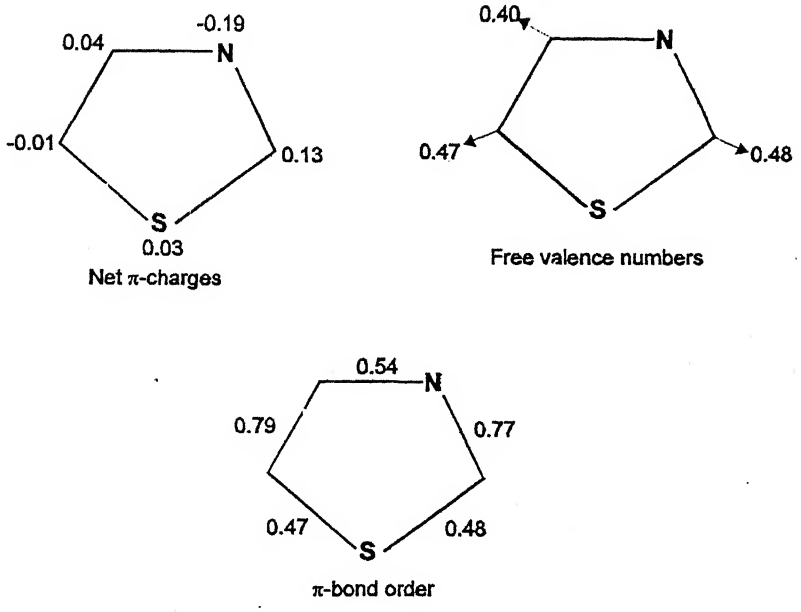


Fig. 2.1: Electronic diagrams for thiazole by HMO method

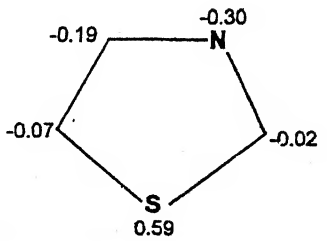


Fig. 2.2: Total electronic net charge of thiazole by *ab initio* method.

simple HMO methods, in accordance with radical localization energies, predict a decreasing order of free radical reactivity of three positions of the ring as 2 > 5 > 4 and the ionic localization energies, in accordance with the net π-charges diagram, predict the decreasing orders of electrophilic and nucleophilic reactivities, respectively, as 5 > 2 > 4 and 2 > 5 >

4. All of these predictions have been confirmed by experiment. The calculated electronic distribution can be used to evaluate the dipole moment of the thiazole: all valence electron methods predict a moment of 1.7-1.8 D with an orientation of -63° relative to the C(2)-C(5) axis, whereas, experimental values are 1.61 D and an angle of -53°.

2.1.2 : Molecular Dimensions :

The size, shape, configuration and conformation of thiazole, benzothiazole and its derivatives have been studied by various theoretical methods, the predictions of which are usually in accordance with experimental data. The relative high thermal stability of thiazole and benzothiazole correlates with calculated bond breaking energies for hydrogen atoms bound to these rings.

The complete determination of the geometrical parameters of the thiazole molecule has been made by a microwave spectroscopic study of thiazole itself and eight isotopically labelled isomers **[18]**. The structure obtained for thiazole is surprisingly close to an average of the structures of thiophene and 1,3,4–thiadiazole **[19]**. From the direction of the quadrupole axis of nitrogen it is concluded that its lone pair is symmetrically placed outside the ring, along the bisector of the angle C(2)-N-C(4).

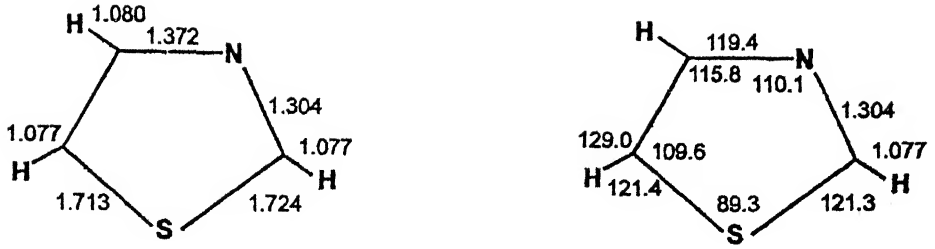


Fig 2.3 : Molecular structure of thiazole; bond lengths (Å) and bond angles (°)

2.1.3 : Ultraviolet Absorption Spectroscopy :

The Ultraviolet absorption spectrum of thiazole was first determined in ethanolic solution by Leandri et al. **[20]** in 1955, then in 1957 by Sheinker et al. **[21]**, and in 1967 by Coltbourne et al. **[22]** in different solvents. The results are summarized in Table 2.2.

The vapour phase UV absorption spectrum of thiazole exhibits two bands at (A) 228 and (B) 206.5 nm. Both bands undergo a red shift of about 3 nm when thiazole is dissolved in hydrocarbon solvents, while the red shift of band (A) increases when the solvent is hydroxylic. The bathochromic shift is typical of $\pi \rightarrow \pi^*$ transitions. In acidic solution, the red shift of band (A) is 9 nm. Methyl substitution does not alter fundamentally the absorption spectrum of thiazole, including a slight bathchromic shift both in vapour phase and in dichloromethane solution, the effect decreasing in the order 4–>5–>2–, with a significant increase in intensity (Table 2.3). Benzothiazole and 2-Methylthiazole exhibit similar behaviour (Table 2.4). Introduction of a functional group is characterized by a more pronounced bathochromic effect (Table 2.5). Quantum mechanical calculations predict UV transitions,

which are in good agreement with the experimental values in case of thiazole and its three-methyl derivatives. A very weak absorption has been detected at 269.5 nm that could correspond to an n → π* transition, predicted by calculation at 280 nm.

2.1.4 : Infrared Spectroscopy :

The study of IR spectrum of thiazole in various physical states (solid, liquid, vapour and solution) **[23]** and of isotopically labeled isomers **[40]**, established the symmetry properties of the main vibrations of the molecule, and the calculation of its normal modes of vibrations defined a force field for it and confirmed quantitatively the assignments given in Table 2.6.

The planar structure of thiazole implies a CS –type symmetry (Fig 2.4), which means all the 18 fundamental vibrations are active in infrared spectroscopy. The IR study of a large number of thiazole derivatives allowed the designing of a Table 2.7, giving the mean vibration frequencies characteristics of C—H bonds [ν(CH), δ(CH), γ(CH)] as a function of substitution pattern.

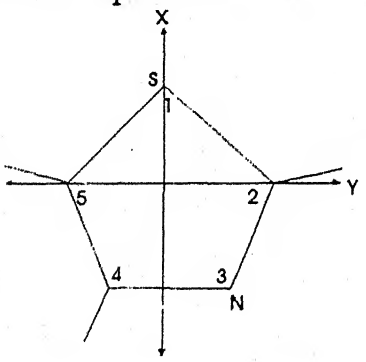


Fig 2.4 : Orientation of x and y axes in the plane of thiazole molecule.

2.1.5 : NMR Spectroscopy :

The nuclear magnetic resonance spectra of thiazoles are remarkably simple and apparently univoque. The proton NMR spectra of thiazole are simple and first order. The deshielding observed can be correlated with the occurrence of a ring current connected with the aromatic sextet of thiazole and the magnetic anisotropy of both sulfur and nitrogen heteroatoms.

The first proton NMR spectrum of thiazole was described by Bak et al. **[25]**. It was followed by a series of works establishing a systematic description of thiazoles, namely the resonance peak assignments, the chemical shifts, and coupling constants as well as the evolution of the spectrum as a function of solvent or temperature **[26-33]**. The signal assignment could be made without ambiguity thanks to the study of various substituted thiazoles, unequivocally synthesized **[34]**, and of 2- and 5-deuteriated thiazole **[27]**. Characteristic proton chemical shifts are given in Table 2.8. For protons bonded to sp^2–carbon atoms, these chemical shifts are located at relatively low field, this deshielding can be correlated to the occurrence of a ring current connected with the "pseudoaromatic" character of thiazole and also to the magnetic anisotrophy of both sulfur and nitrogen heteroatoms. Furthermore, it seems that this latter atom plays a preponderant role in the chemical shifts observed for the protons of benzene (7.30 ppm) on one hand, and those in the α-position of thiophene (7,30 ppm) and pyridine (8.60 ppm) on the other hand. More sophisticated analytical methods have

been used to define the characteristics of the ring current: from empirical calculation **[35]**, from experimental data **[36]**, or attempts to determine the "aromaticity" of the ring on the basis of this ring current **[37]**. These studies show the similarity between ring currents in thiazole and thiophene, and more especially, pyrrole **[37,35]** and the absence of correlation with resonance energies **[36]**. In a first analysis no correlation could that these substituents show characteristic group frequencies **[38]** that make possible the distinction between vibrations essentially originating in the thiazole ring and those of substituents.

2.1.6 : Thermodynamic Aspects :

(a) Melting And Boiling Points :

The thermodynamic study of thiazole has led not only to the determination of important practical data, but also to the discovery of thiazole self association in the liquid state.

The vapour-liquid equilibrium of highly purified thiazole has been determined from 100 to 760 mm Hg and the experimental data correlated by the Antoine equation **[39,40]**, with P in mm Hg and t in °C (equation 1)

$$\log_{10} P = 7.14112 - 1424.800/(t + 216.194) \qquad .....(1)$$

2-Methylthiazole behaves similarly fitting the Antoine equation (2) **[41]**

$$\log_{10} P = 7.04109 - 1406.419/(t + 209.257) \qquad .....(2)$$

The normal boiling pints of pure thiazole and 2-Methylthiazole are respectively t_{760}=118.24 and 128.49°C.

(b) Solubility :

Thiazole is entirely soluble in water at room temperature but when distilled exhibits an azeotropic mixture having, under a pressure of 750 mm Hg, a molar fraction of water of 0.72 and an equilibrium temperature of 65°C. Thus, the dehydration of thiazole cannot usually be performed by simple distillation; one must use dehydrating agents. Thiazole is entirely miscible with most organic solvents. At lower temperatures the binary mixtures exhibits varied behaviour; with cyclohexane, carbon tetrachloride and benzene for example **[42,43]**, one observes eutectic mixtures having, respectively, the following characteristics (m.p./°C, molar fraction of thiazole): -38.4/0.82; -60.8/0.46; and -48.5/0.70. The first system is characterized by a partial miscibility of the liquid phases, the second one is unstable with incongruent melting points at -54 and -52.8°C and the third is a simple eutectic mixture. Observed deviations from ideality for the solutions are attributed to thiazole self-association. The constants of self-association have been estimated for cyclohexane solution of thiazole (K_{assoc} = 5 at 6.5°C) and for benzene solutions of thiazole (K_{assoc} = 3 at 5.5°C). Similarly, molar excess functions have been determined for various thiazole–solvent binary mixtures (Table 2.9). For cyclohexane the excess enthalpy HE is positive and large, whereas, for solvents with aromatic character it is low, and even negative in case of pyridine.

A conclusion of all these thermodynamic studies is the existence of thiazole-solvent and thiazole-thiazole associations. These associations are confirmed by the result of viscosimetric and diffusiometric studies on thiazole and binary mixtures of thiazole and cyclohexane or CCl_4. In all case of cyclohexane, the solvents seems to destroy some thiazole self-associations (aggregates) existing in the pure liquid, whereas in the case of carbon tetrachloride there is association of two thiazole molecules with one solvent molecule. The most probable mode of the self-association of thiazole is of the n-π type from the lone electron pair of the nitrogen of one molecule to the LUMO of the other.

(c) Stability and Stabilization :

(i) The thermal stabilities of thiazole and of some aryl and benzo derivatives have been determined and the pyrolysis temperatures are reported in Table 2.10 **[44]**). They have been correlated with the energy of the LUMO whereas, there is no correlation with calculated resonance energies.

(ii) Aromaticity :

Many physiochemical properties of thiazole and benzothiazole are consistent with the aromatic character of the heterocyclic ring. The geometrical structure of thiazole is very close to the average of that of thiophene and 1, 3, 4-thiadiazole suggesting an aromaticity of the some order for three molecules. The microwave spectrum of thiazole exhibits practically no inertial defects, which implies entire coplanarity of the eight atoms of the ring, in accordance with an important

cyclic conjugation of their p_z orbitals. The important ^1H NMR deshielding of ring protons for thiazole and benzothiazole has been correlated with the occurrence of a ring current connected with the aromatic character of the thiazole ring (and also with magnetic anisotropy of both sulfur and nitrogen heteroatoms).The important magnetic susceptibility anisotropy of thiazole is also compatible with its aromatic character. The large intensity of the molecular ion in the mass spectrum of thiazoles and benzothiazoles is associated with the aromatic character of these rings.

2.2 : Experimental :

(a) Materials Employed :

2-Aminothiazole, benzothiazole, and 2-methyl benzothiazole were procured from Aldrich Chemical Company, U.S.A. and used as such $PtCl_2$ and chemicals 2-aminothiazole, benzothiazole, and 2-methyl benzothiazole were obtained from TOKYO KASEI Organic Chemical, Japan and B. D. H England . Distilled water used in all the operation.

(b) Preparation of the coordination Compound :

(i) Preparation of the Coordination Compound [Pt(2-ATZ)$_2$Cl$_2$] :

A mixture of $PtCl_2$(500mg) and ligand 2-Amino thiazole (1gm) in water and methanol (50ml) was refluxed at 80^{0}C 5-6 hours until it became a clear brownish colour solution. This volume was reduced to 5ml and treated with methanol. The resulting brownish crystals were collected and washed well

with ethanol and acetone. The analytical data are given in Table 2.11.

(ii) Preparation of the Coordination Compound $[Pt(BTZ)_2Cl_2]$:

A mixture of $PtCl_2$(500mg) and ligand Benzothiazole (1gm) in water and methanol (50ml) was refluxed at 80°C 5-6 hours until it became a clear white colour solution. This volume was reduced to 5ml and treated with methanol. The resulting dirty-white crystals were collected and washed well with ethanol and acetone. The analytical data are given in Table 2.11.

(iii) Preparation of the Coordination Compound [Pt(2-Methyl $BTZ)_2Cl_2$] :

A mixture of $PtCl_2$ (500mg) and ligand 2-Methyl benzothiazole (1gm) in water and methanol (50ml) was refluxed at 80°C 5-6 hours until it became a clear yellowish colour solution. This volume was reduced to 5ml and treated with methanol. The resulting light-brown crystals were collected and washed well with ethanol and acetone. The analytical data are given in Table 2.11.

The general reaction for the preparation of coordination compounds of platinum is as follows :

$$[Pt\ (Cl)_2] + 2L \xrightarrow[H_2O]{CH_3OH} [Pt\ (L)_2(Cl)_2]$$

where L = 2-Aminothiazole, benzothiazole, and 2-methyl benzothiazole.

(c) Analysis of the Constituents Elements :

(i) Carbon, Hydrogen, Nitrogen and Sulphur present in the investigated complexes were estimated micro-analytically.

(ii) Estimation of Pt :

For the estimation of platinum as ammonium chloroplatinate, dissolved the compound in 5ml of concentrated hydrochloric acid and 20ml of hot water, and then add gradually an equal bulk of half-saturated ammonium chloride solution. Allow to stand for 8 hours, filtered off the precipitate, wash it with ammonium chloride solution, and finally twice with cold water. Transfered the filtered paper and precipitate to a Main-Smith crucible, heat extremely slowly at first, and ultimately raise to a bright red heat. Repeated heating cooling and weighting were carried out until weight obtained constant.

(d) Physical Methods :

(i) Molecular Weight Determination :

Molecular weight determination of the synthesized complexes were made by Rast's Method.

(ii) Magnetic Susceptibility Measurements :

The magnetic susceptibility measuerments were made at room temperature by the Gouy Method. A magnetic field strength of 8500 guass was employed. The apparatus was calibrated using cobalt mercury thiocynate $Hg[Co(NCS)_4]$. The diamagnetic corrections were computed using Pascal's constant **[45,46]**. For calculations of effective magnetic moment following equation has been used.

Effective magnetic moment (μ eff) = 2.84 ($X^{corr}m$ T)^{½}, where T = temperature in absolute scale and X_m = corrected molar susceptibility.

(iii) Conductance Measurement :

Conductance was measured in analytical grade methanol using dip type cell with the help of a Philips Conductivity Bridge.

(iv) Infrared Spectral Measurement :

Infrared spectra (4000-600 cm^{-1}) of the uncoordinated ligands and of the complexes were recorded as Nujol Mulls supported between sodium chloride platex (rock salt regions) on a Perkin Elmer Spectrum RXI Spectrometer.

(v) ^{1}HNMR Spectral Measurement :

^{1}HNMR Spectra of the synthesized compounds will be recorded on AC 300F Spectrometer (300MHz) using TMS as an internal standard.

(vi) Electron Spin Resonance Spectra :

Electron spin resonance spectra of the complexes were recorded at room temperature on a Varium E-3 spectrometer using powdered sample at the microwave frequency 9.53GHz. The 'g' values were calculated using the given equation.

$$g = \frac{714.44 \times \sqrt{(GHz)}}{H\ (G)}$$

where $\sqrt{(GHz)}$ = microwave frequency in GHz at which sample operated, and H(G) = field in Gauss for the sample.

2.3 : Properties of the Complexes :

The analytical and physical data of the ligand and its metal complexes are given in Table no 2.11. The complexes are non-hygroscopic and stable at room temperature. The solubility of complexes $[Pt(2\text{-}ATZ)_2Cl_2]$, $[Pt(BTZ)_2Cl_2]$ and $[Pt(2\text{-methyl-}BTZ)_2Cl_2]$ in different solvent are shown in Table no 2.13. They are soluble in DMF and DMSO, slightly soluble in dioxane and insoluble in other organic solvent. The colour of all these complexes are shown in Table no 2.12. They do not possess sharp melting points.

2.4 : Result and Discussion :

(a) Magnetic Measurement :

The magnetic values of synthesized complexes measured at room temperature. An observation shows that the magnetic moment values of the complexes $[Pt(2\text{-}ATZ)_2Cl_2]$, $[Pt(BTZ)_2Cl_2]$ and $[Pt(2\text{-methyl-}BTZ)_2Cl_2]$ are zero. Hence, all the complexes are diamagnetic and also the square-planar geometry of compounds are evident from their diamagnetic nature.

(b) Conductance Measurement :

The analytical and physical data of the ligand and its metal complexes are given in Table no 2.11. The values of molar conductance are in the range 0.052-0.058 Ω^{-1} cm^2 mol^{-1} suggesting non-electrolyte nature of the synthesized complexes.

(c) Infrared Spectral Measurement :

The ligand 2-Aminothiazole possesses two possible donor sites; amino nitrogen and sulfur of the thiazole ring. Further the amino group involved in coordination through N atom.

Coordination through N of the amino group invariably results in negative shift in υNH_2 (3105) by at least 50 cm^{-1}(3055 cm^{-1}). The ligand band at 780-740 cm^{-1} (C=S) does not undergo any shift in complex, indicating non-involvement of sulfur atom. The ligand benzothiazole possesses only two donor sites; tertiary cyclic nitrogen and sulfur moiety. The IR frequency of tertiary cyclic nitrogen of thiazole ring (1375 cm^{-1}) undergoes a negative shift of 52 cm^{-1} (1323cm^{-1}), thereby, suggesting that the cyclic nitrogen of this ligand is involved in the coordination, whereas, ligand band at 780-740 cm^{-1} (C=S) does not undergo any shift in complexes, indicating non-involvement of sulfur atom in coordination. The ligand 2-methyl benzothiazole has only two donor sites ; tertiary cyclic nitrogen and cyclic sulfur. The IR frequency of tertiary cyclic nitrogen of 2-methyl benzothiazole ring (1375 cm^{-1}) undergoes a negative shift of 60 cm^{-1} (1315 cm^{-1}), thereby, suggesting that the cyclic nitrogen of this ligand is involved in the coordination, whereas, ligand band at 780-740 cm^{-1} (C=S) does not undergo any shift in complexes, indicating non involvement of sulfur atom in coordination.

(d) Electron Spin Resonance Spectra :

The electron spin resonance data for the synthesized complexes under this investigation are given in Table 2.15.

The recorded 'g' values in the range 1.982-1.988 are constant. The electronic spectral bands of the complexes (Table 2.16) were assigned according to the literature **[47,48]**.

The molecular orbital approach was used to explain the structure of square-planar complexes of the d^8 elements. The metal orbitals involved in σ-bonding in square–planer complexes are the nd_{z^2}, $nd_{x^2-y^2}$ (n+1)s, $(n+1)P_x$ and $(n+1)P_y$. Nevertheless, judging from the values of the overlap integrals, ndx^2-y^2 (n=1)s, $(n+1)P_x$ and $(n+1)P_y$ account for most of the σ-bonds, and ndz^2 makes only a minor contribution. The most important π–molecular orbital and a combination of π–orbitals of the ligands.

The correlation of the bands observed in the electronic spectra for the studied complexes with those of $[M(CN)_4]^{2-}$ [$M=pt^{II}$] prompted us to assume the following assignments (Table 2.16) $^1A_{1g} \rightarrow {}^1A_{2g}[b_{2g}(\pi^*) \rightarrow b_{1g}(\sigma^*)]$, (d-d); $^1A_{1g} \rightarrow {}^1B_{1g}[b_{2g}(\pi^*) \rightarrow a_{1g}(\sigma^*)]$, (d-d); $^1A_{1g} \rightarrow {}^1E_g[e_g(\pi^*) \rightarrow b_{1g}(\sigma^*)]$, (d-d); $^1A_{1g} \rightarrow {}^1B_{1u}$ $[b_{2g}(\pi^*) \rightarrow a_{2u}(\pi^*)]$, (C.T); $^1A_{1g} \rightarrow {}^1E_u[e_g(\pi^*) \rightarrow a_{2u}(\pi^*)]$, (C.T).

The relation between the bands in the present complexes and the described for the typical complexes $[M(CN)_4]^{2-}$ leads to the conclusion that all the new complexes have the same square-planar geometry.

(e) NMR Spectroscopy :

The spectrum of Pt (II) with 2-Aminothiazole complex dissolved in D_2O consists of a broad NH_2 signal at 5.38ppm. At later times, or raising the pH, the NH_2 protons exchange completely. Spectrum of aforesaid compounds shown into figure. The methyl proton spectrum consists of doublet, due to N-H coupling, flanked by symmetric doublet ^{195}Pt side bands.

Effect of Coordination on Chemical Shifts :

Coordination to Pt invariably produces substantial downfield shifts for ligand protons. The extent of the downfield shift is similar to corresponding protonation shifts and decreases similarly with distance from the coordination site. Among the methyl-substituted glycines, chelation produces a downfield shift about 80% as great as that resulting from single protonation. Not surprisingly, CH_2 signals of N-coordinated glycinates are shifted downfield only half as much as those of chelated glycinates, but are much more affected by protonation of the free CO_2^- group.

Such observations are the basis for concluding that the α-carboxyl group, rather than the β-carboxyl group, is involved in chelation top Pt in complexes of L-aspartate. For those complexes, the methane proton is more affected by complexing, while the methylene protons are more affected by subsequent titration of the uncomplexed carboxyl. For S-methylcysteine, and methionine, the effect of protonation of NH_2 and CO_2^- on CH_3 shifts is not large, but coordination of NH_2 to Pt produces a downfield shift comparable to the effect of protonation or complexing N for N-CH_3 groups.

Differences in chemical shifts for all ligand protons were negligible for the following pairs of isomers: *trans*-Pt(L-Asp)$_2$ and Pt(L-Asp)(D-Asp), *trans*-Pt(L-Glu)$_2$ and Pt(L-Glu)(D-Glu), and *cis*- and *trans*-Pt(L-Dap)$_2$. For Pt(L-Hist)$_2$, chemical shifts of *cis* and *trans* isomers are very similar for corresponding protons of corresponding species, except for C_2-H whose environment in the two isomers varies significantly.

Platinum-Proton Coupling and Coordination :

The presence or absence of Pt side bands provides a clear identification of the site of coordination in these complexes. In general, three-bond Pt-N-C-H couplings are between 10 and 60 Hz. Coupling through four bonds is negligibly small, except for C_4-H of histidine which is coupled through four bonds nearly as strongly as is C_2-H through three bonds **[49]**.

Not surprisingly, in view of the known strong tendency of Pt (II) to coordinate sulfur, $J_{Pt\text{-}S\text{-}C\text{-}H}$ values are generally among the largest values observed. A small, but noteworthy, variation in $J_{Pt\text{-}CH_3}$ was observed for 1:1 complexes of both S-methylcysteine and methionine. Starting with $Pt(AA)Cl_2$, replacement of the Cl *cis* to the N of AA by NH_3 decreases $J_{Pt\text{-}CH_3}$ from ~55 to ~45 Hz, while replacement of both Cl's by NH_3 or ethylenediamine has a smaller effect (~55 to ~50 Hz).

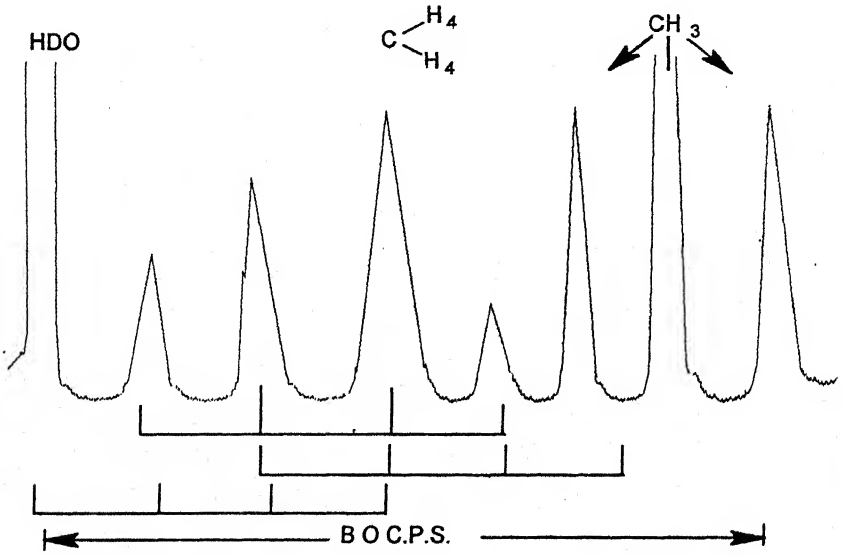


Fig.2.5: Proton nmr spectrum of the sarcosine complex $K[Pt\text{–}(NDCH_3CH_2CO_2)Cl_2]$ in D_2O.

The down field shifts of proton signals indicates bonding of the ligand of the metal atom. From NMR spectra no specific information could be drawn as no specific position of neighbouring proton signal adjacent to donor atoms could be located.

2.6 : Summary :

The mixed ligand complexes $[PtL_2Cl_2]$ where (L = 2-amino thiazole, benzothiazole and 2-methyl benzothiazole), have been prepared by the interaction of parent compound $PtCl_2$ with ligand. The complexes are characterized by elemental analysis, magnetic measurement, electron spin resonance and infrared spectral studies containing Pt (II) d^8 configuration. All the complexes are diamagnetic suggesting square planner geometry. It is observed that:

(i) The DMF solution of the synthesized compounds are non-conducting.

(ii) All the complexes contain low spin d^8 configuration.

(iii) The reflectance spectra of the complexes display a shoulder at 340-430 nm, which is attributable to transition

$A_{1g} \longrightarrow A_{2g}$

(iv) All the compounds are thermally stable upto 200^0C.

(v) All the complexes show anticancer activity.

Table 2.1 :

Localization Energies (β units) for Thiazole By HMO Method

Position				
	2	**4**	**5**	**Reactivity**
Radical	1.99	2.51	2.01	2>5>4
Electrophilic	2.19	2.55	2.01	5>2>4
Nucleophilic	1.79	2.47	2.01	2>5>4

Table 2.2 :

Ultraviolet Absorption Spectrum of Thiazole in the Vapour Phase and in Different Solvents.

Solvent	**Band A**		**Band B**	
	λ (nm)	**Log ε**	**λ (nm)**	**log ε**
Vapour phase	228	-	206.5	-
n –Hexane	231-2	-	209	-
Cyclohexane	231-2	-	209-10	-
Dichloromethane	234	3.05		
Methanol	232-3	-	-	-
Ehanol	233	3.57	207.5	3.41
	232-3	3.52-3.59	-	-
	240	3.59	-	-
Water	233	3.53	205	3.34
Water, pH 13	233	3.54	-	-
Water, pH 5	235	3.51	-	-
1 *N* HCl, pH 0	237	3.61	-	-
1.8 *N* HCl	237	3.59	-	-

Table 2.3 :

UV Absorption Spectra of Thiazole and Monomethyl Derivatives

	Vapour phase λ_{max} (nm)	Solution (CH_2Cl_2) λ_{max} (nm)	log ε
Thiazole	228	234	3.05
2-Methyl	231.6	236	3.60
4-Methyl	237.4	242.5	3.53
5-Methyl	234.7	241.8	3.48

Table 2.4 :

UV Absorption Spectra (Alcohol) of Benzothiazole and 2-Methylbenzothiazole.

	λ (nm)	log ε	λ (nm)	log ε	λ (nm)	log ε
Benzothiazole	294	3.13	285	3.22	252	3.76
2-Methyl	293	3.14	283	3.20	252	3.87

Table 2.5 :

UV Absorption Spectra (Alcohol) of Some Typical Derivatives of Thiazole

	λ_{max} (nm)	log ε		λ_{max} (nm)	log ε
Thiazole	233	3.59	2-Bromo	247.6	3.69
2-Methoxy	235	3.67	5-Bromo	247.4	3.61
2-Ethoxy	237.5	3.68	2-Iodo	253	-
5-Ethoxy	255.2	3.62	2-Nitro	308	3.68
2-Chloro	244.5	3.72	5-Nitro	298.5	3.68
5-Chloro	245.6	3.46	2-Dimethylamino	265.3	3.96

Table 2.6 :

Thiazole Vibration Frequencies and Assignments

Symmetry mode	Experimental frequencies ν (cm^{-1})	Theoretical frequencies	Theoretical assignments	Experimental assignments
A′	3126	3125	$97\nu(C^5H)$	$\nu(C^5H)$
		3091	$97\nu(C^4H)$	$\nu(C^4H)$
	3092	3087	$99\nu(C^2H)$	$\nu(C^2H)$
	1478	1477	$58\nu(C=C)+28\nu(C-N)$	ω_1
	1378	1393	$60\nu(C=N)+25\nu(C=C)$	ω_2
	1319	1326	34ν (C-N)$+23\nu$ (C=C) $+22\nu$ (C=N)	ω_3
	1326	1248	27δ (C^2H)+ 27δ (C^4H)$+17\delta$ (C^5H)	δ(CH)
	1122	1105	$41\delta(C^4H)+7\delta(C^5H)+7\delta(C^2H)$	δ(CH)
	1041	1047	$72\delta(C^5H)+24\delta(C^4H)$	δ(CH)
	862	875	60δ(N)$+38\nu$(C-S)	ω_4
	811	817	93ν(C-S)	ω_5
	753	749	61ν(C-S) $+33\delta$(N)	ω_6
	610	607	65δ(N) $+33\nu$(C-S)	ω_7
A″	877	877	$86\gamma(C^4H)+11\gamma(C^2H)$	$\gamma(C^4H)$
	795	804	$68\gamma(C^2H)+28\gamma(C^4H)$	$\gamma(C^2H)$
	718	704	$98\gamma(C^5H)$	$\gamma(C^5H)$
	602	603	22τ(C=C)$+44\tau$(C-N)$+22\tau$(C=N)	Γ_1
	467	474	46τ(C-S)$+12\tau$(C=C)	Γ_2

δ(N) = angular bending of the ring; τ(C-X) = torsion of the C-X bond.

Table 2.7 :

Mean Positions of the C—H Vibrations of Thiazole Derivatives

Position of substituent	ν(C^5H)	ν(C^4H)	ν(C^2H)	δ (C^2H)	δ (C^4H)	δ (C^5H)	γ C^4H)	γ (C^2H)	γ (C^5H)
2	3118±8	3087±7	—	—	1164±37	1057±11	874±8	—	702±21
4	3121±11	—	3085±9	1218±20	—	1091±46	—	808±6	722±4
5	—	3089±9	—	1226±8	1109±7	—	851±4	785±2	—
2,4	3120±12	—	—	—	—	1083±55	—	—	707±32
2,5	—	3088±13	—	—	1149±12	—	839±2	—	—
4,5	—	—	3085±7	1215±29	—	—	—	785±3	—

Table 2.8 :

Proton Chemical Shifts of Thiazole

ΔH(2)	δH(4)	ΔH(5)	Solvent
-2.31	-1.70	-1.35	b
8.68	7.83	7.19	C_6H_{12}
10.0	8.45	8.23	CF_3COOH
9.15	7.97	7.75	DMSO
9.00	7.93	7.65	Acetone
8.70	7.84	7.22	C_6H_6
8.77	7.86	7.25	CCl_4
8.88	7.98	7.41	$CDCl_3$
7.72	6.62	6.23	TFA
8.55	7.54	7.05	$CDCl_3$
	7.86	7.27	CCl_4

(Chemical shifts are expressed in δ units; ppm of applied magnetic field with internal TMS peak as reference. In case b, H_2O peak is external reference).

Table 2.9 :

Partial Molar Excess Enthalpy at Infinite Dilution (H^E) of Thiazole in Various Solvents at 318.15 K

Solvent	H^E (J mol^{-1})	Solvent	H^E (J mol^{-1})
C_6H_{12}	10199	C_6F_6	2801
C_6H_6	1200	Thiophene	401
CCl_4	3386	Pyridine	-301

Table 2.10 :

Pyrolysis Temperature of Some Thiazoles and Benzothiazoles

Thiazole	**T (°C)**	**Thiazole**	**T (°C)**
Thiazole	530	2,4-Bis(p-biphenylthiazole)	510
2,4-Diphenylthiazole	431	Benzothiazole	556
2-Phenyl-4-biphenylthiazole	442	2-Methylbenzothiazole	446
4-Phenyl-2-biphenylthiazole	452	2-Phenylbenzothiazole	504

Table 2.11 :

Analytical and Electronic Spectral Data of Pt (II) Complexes

Compound	Found (Calc.)%						Mol.Wt. Found (Calc.)
	M	C	H	N	S	Cl	
$[Pt(C_3H_4N_2S)_2Cl_2]$	41.43 (41.84)	15.26 (15.46)	1.47 (1.73)	11.943 (12.02)	13.46 (13.75)	14.80 (15.21)	464.96 (465.37)
$[Pt(C_7H_5NS)_2Cl_2]$	35.98 (36.37)	31.03 (31.35)	1.32 (1.88)	5.00 (5.22)	11.52 (11.96)	13.00 (13.22)	535.97 (536.46)
$[Pt(C_8H_7NS)_2Cl_2]$	34.01 (34.56)	33.76 (34.05)	2.10 (2.50)	4.34 (4.96)	12.94 (13.36)	12.23 (12.56)	564.12 (564.51)

Table 2.12 :

Colour and % Yield of the Complexes

S.No.	Compound	Colour	%Yield
1.	$[Pt\ (2\text{–}ATZ)_2Cl_2]$	Brown	60
2.	$[Pt\ (BTZ)_2Cl_2]$	Dirty–white	58
3.	$[Pt\ (2\text{–methyl BTZ})_2Cl_2]$	Light–brown	64

Table 2.13 :

Solubilities of The Complexes in Different Solvents

S.No. Compound	DMF	DMSO	EtOH	MeOH	Dioxane	Ethylacetate
1. $[Pt(2\text{–}ATZ)_2Cl_2]$	Soluble	Soluble	Insoluble	Insoluble	Sparingly soluble	Insoluble
2. $[Pt(BTZ)_2Cl_2]$	Soluble	Soluble	Insoluble	Insoluble	Sparingly soluble	Insoluble
3. $[Pt(2\text{–methyl BTZ})_2Cl_2]$	Soluble	Soluble	Insoluble	Insoluble	Sparingly soluble	Insoluble

Table 2.14 :

Important IR Spectral Bands and Their Assignments

S.No	Compound	υ (NH_2)	υ C-S(cyclic)	υ N(cyclic)
1.	[Pt(2-aminothiazole)$_2$Cl$_2$]	3055 cm^{-1}	unchanged	unchanged
2.	[Pt(benzothiazole)$_2$Cl$_2$]	-	(780-740cm-1)	(1375cm^{-1})
3.	[Pt(2-methylbenzothiazole)$_2$Cl$_2$]	-	unchanged (780-740cm^{-1})	1323 cm^{-1}
			unchanged (780-740cm^{-1})	1315 cm^{-1}

Table 2.15 :

Electronic Spectral Data of the Complexes

$[PtCl_2(L)_2]$	
21700	$^1A_{1g} \rightarrow {}^1A_{2g}[b_{2g}(\pi^*) \rightarrow b_{1g}(\sigma^*)]$
265001	$^1A_{1g} \rightarrow {}^1B_{1g}[b_{2*}(\pi^*) \rightarrow a_{1g}(\sigma^*)]$
304001	$^1A_{1g} \rightarrow {}^1E_g[e_g(\pi^*) \rightarrow b_{1g}(\sigma^*)]$

Table 2.16 :

Electronic Spectra of MCl_2L_2 Chelates

Transition	**$[PtL_2Cl_2]$**
d-d	
$A_g \rightarrow {}^3B_{1g}(x^2-y^2 \rightarrow xy)$	
$^1A_{1g} \rightarrow {}^1B_{1g}(x^2-y^2 \rightarrow xy)$	18, 180 (3.45)
$^1A_g \rightarrow {}^3B_{3g}(xz \rightarrow xy)$	
$^1A_g \rightarrow {}^1B_{3g}(xz \rightarrow xy)$	17, 480 (3.57)
M→L charge transfer	
$^1A_{1g} \rightarrow {}^1B_{2u}[xz \rightarrow L(\pi^*)]$	17, 480 (3.57)
$^1A_g \rightarrow {}^1B_{3u}[yz \rightarrow L(\pi^*)]$	28, 000 (3.90)
M→L charge transfer	
$^1A_{1g} \rightarrow {}^1B_{2u}\ {}^1B_{3u}[L(\pi) \rightarrow xy]$	35, 710 (4.12)
$^1A_g \rightarrow {}^1B_{2u}\ {}^1B_{3u}[L(\sigma) \rightarrow xy]$	41, 840 (4.52)
L→L*	
$^1A_g \rightarrow {}^1B_{2u}$	29, 940 (4.01)
$^1A_g \rightarrow {}^1B_{1u}$	38, 170 (4.28)

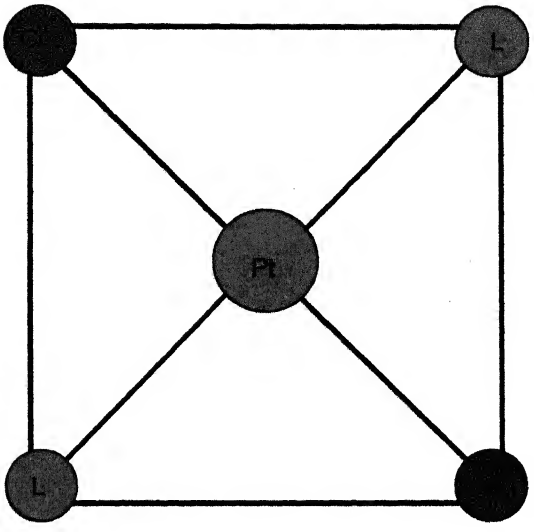


Fig .2.6 : Proposed square planar structure of $[Pt(L)_2Cl_2]$ (where L= 2-aminothiazole, benzothiazole and 2-methyl benzothiazole)

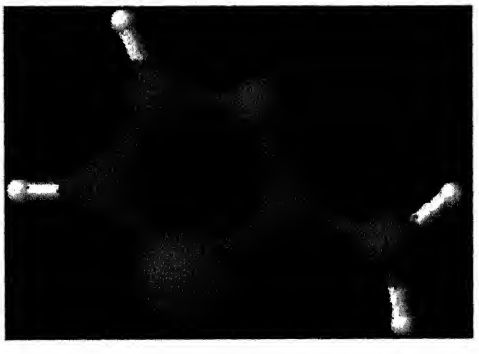


Fig. 2.7 : 2-Aminothiazole

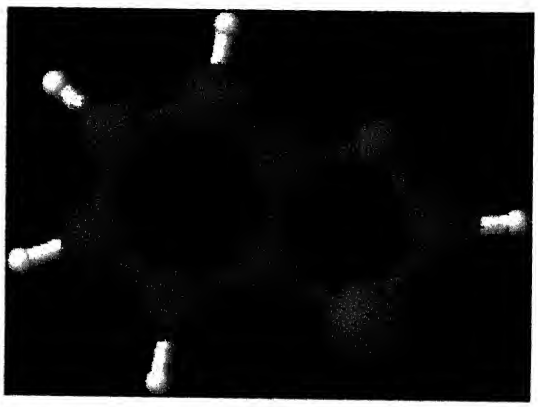
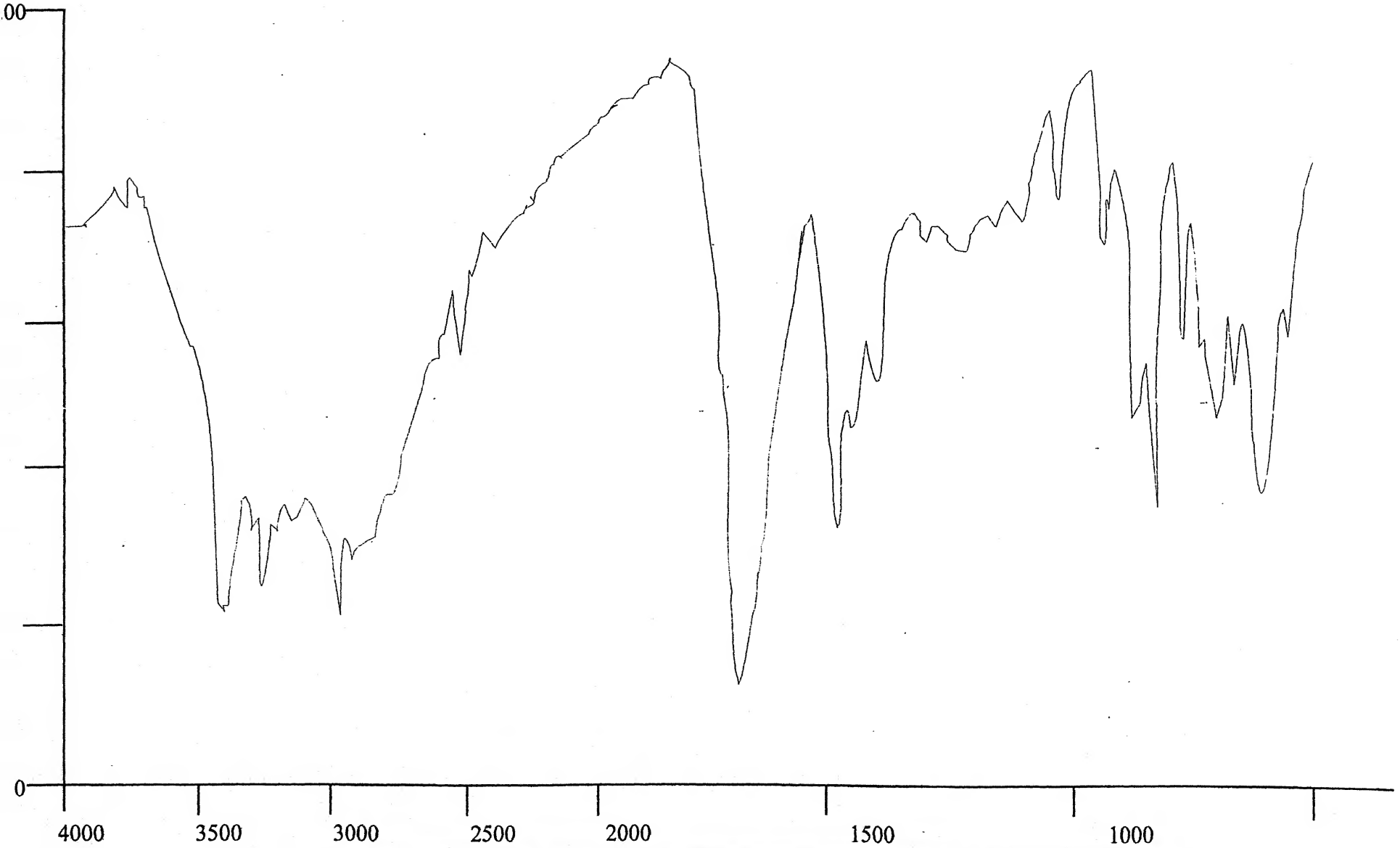
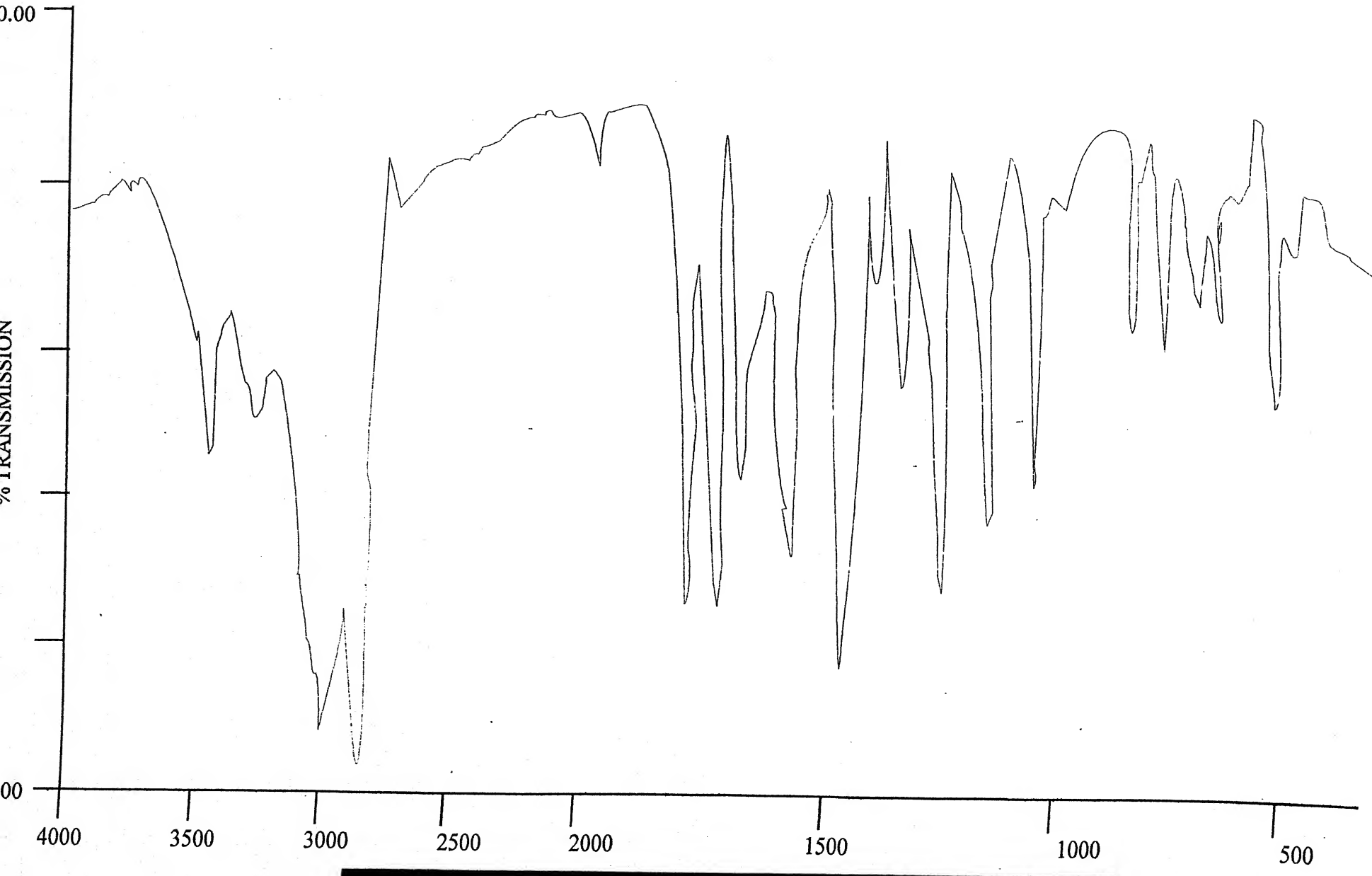


Fig .2.8 : Benzothiazole

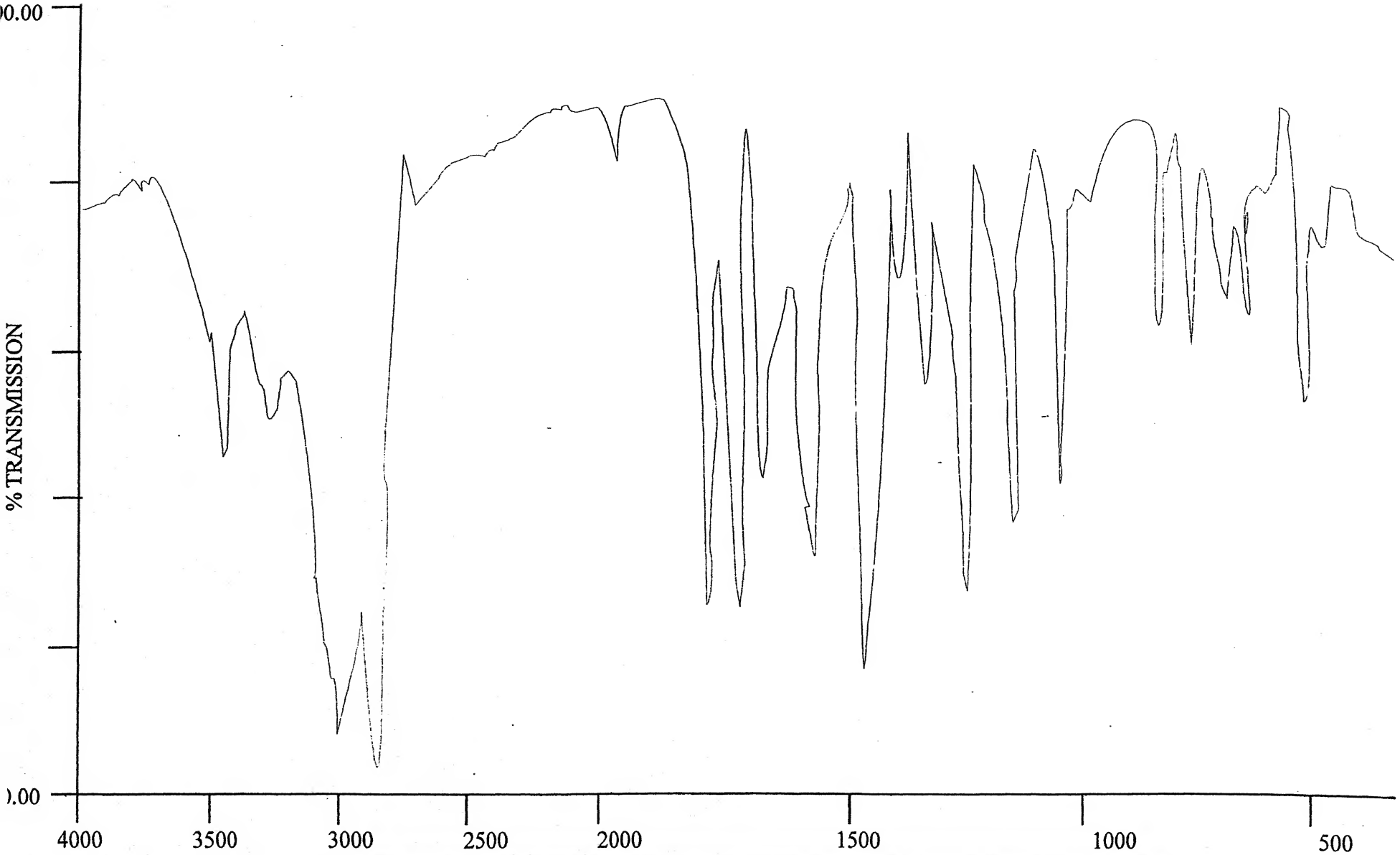
Fig .2.9 : 2-Methyl benzothiazole



INFRARED SPECTRUM OF [illegible]



INFRARED SPECTRUM OF $[Pt(BTZ)_2Cl_2]$



INFRARED SPECTRUM OF $[Pt(BTZ)_2Cl_2]$

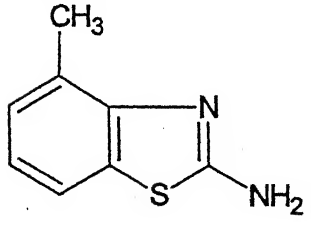
CHAPTER-III

CHAPTER -III

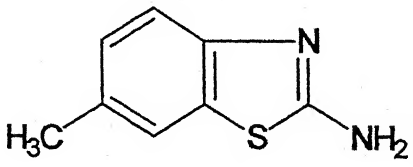
3.1 : Introduction :

In chapter II, synthesis and physiochemical studies of some mixed ligand complexes of platinum with some substituted benzothiazoles have been discussed. As a part of our programme to synthesize and characterize some neutral mixed ligand complexes of divalent platinum, studies have been extended using some more benzothiazoles derivatives.

In recent years, a great deal of interest has been shown to the study of neutral mixed-ligand complexes of platinum having d^8 electronic configuration. No attempts have been made so for to isolate complexes of divalent platinum with some substituted 2-aminobenzothiazoles. We, therefore, report here the first synthesis of neutral mixed ligand complexes of platinum with 2-Amino–4–methylbezothiazole and 2-Amino-6-methylbezothiazole. The present chapter describes the results of such studies.



2-Amino-4-methylbenzothiazole



2-Amino-6-methylbenzothiazole

(a) General Survey of Reactivity of Thiazole :

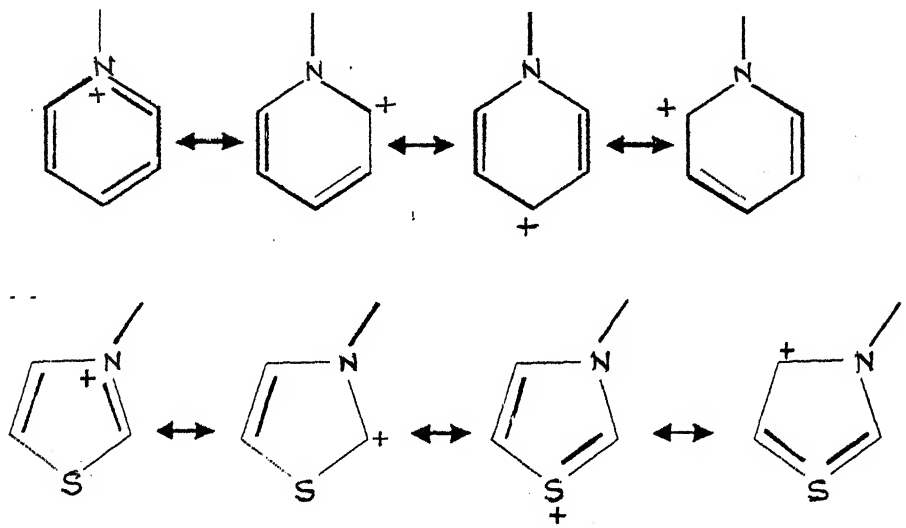
(i) Reactivity of Neutral Thiazole :

Thiazole may be considered to be derived from benzene by replacing a CH group with a nitrogen atom and a CH=CH group at position 3,4 of the corresponding pyridine with a sulfur atom. The

chemistry of the thiazole, therefore, shows similarities to those of both pyridine and thiophene. Thus, electrophilic reagents attack the lone pair on nitrogen but do not attack that on sulfur. The three carbon atoms of the ring behave quite differently, taking into account the complete dissymmetry of the structure, which is mirrored in its electronic distribution. The whole reactivity of the ring in nucleophilic, electrophilic and radical reactions is well explained by data from MO calculations, and the aromatic character of the molecule, which appears in its chemical behaviour is also demonstrated by many physico-chemical properties. The fused ring of benzothiazole exhibits a similar chemical reactivity in good agreement with theoretical calculations.

The most striking analogy between thiazole and pyridine results from the presence, in both the molecules, of a ring N atom, contributing two σ-bonds to the σ-framework of the molecule and one $2p_z$ electron to the π-system, while a lone pair remains localized on the nitrogen atom, described by an sp^2-hybrid orbital, the axis of which lies in the σ-plane and is directed along the bisector of the C–N–C angle. The lone pair of thiazole is, however, less reactive than that of pyridine, due to the enhanced aromatic character of the latter ring which is responsible for a more effective stabilization of the (incipient to integral) positive charge developing on the nitrogen atom is along the reaction path (Scheme 1). Table 3.1 shows three sets of physio-chemical data that illustrate this difference. These are; (i) the thermodynamic basicity, which is three orders of magnitude

lower for thiazole than for pyridine; (ii) the enthalpy of reaction with BF_3 in nitrobenzene solution, which is 10% lower for thiazole; (iii) the specified rate of reaction with methyl iodide in acetone at 40°C, which is about 50% lower for thiazole and which in DMSO at 25°C is of the order of 1/21that of pyridine.



Scheme 1

(ii) Basicity of Thiazoles :

The pK_a values of some representative thiazoles and the free enthalpy of dissociation of their conjugate acids is shown in Table 3.2. Alkyl groups are weakly base strengthening due to their $+I$ effect, which decreases in the order 2-, 4-, 5-alkyl, in agreement with the net π charge on the nitrogen calculated by various theoretical methods. A conjugated +M 2-amino group markedly enhances the basicity while the introduction into the 5-position of a strongly –M nitro group results in a large decrease of the pK_a. A fused benzene ring has little effect on the basicity of the nitrogen atom. The variation of pK_a in both 2- and 4-alkyl-

substituted thiazoles after the α-series of Ingold (Me, Et, Pr^i, Bu^t) shows that both a *+I* effect activating effect and a steric deactivating *ortho* effect have to be taken into account.

3.2 : Experimental :

(a) Materials Employed :

2-Amino-6-methyl benzothiazole and 2-amino-4-methyl benzothiazole were procured from Aldrich Chemical Company, U.S.A. and used as such $PtCl_2$ and chemicals 2-amino-6-methyl benzothiazole and 2-amino-4-methyl benzothiazole were obtained from TOKYO KASEI Organic Chemical, Japan and B.D.H England. Distilled water used in all the operation.

(b) Preparation of the Coordination Compound :

(i) Preparation of the Coordination Compound [Pt (2-Amino-6-methyl BTZ)$_2$Cl$_2$] :

A mixture of $PtCl_2$ (500mg) and ligand 2-amino-6-methyl benzothiazole (1gm) in water and methanol (50ml) was refluxed at 80^{0}C 5-6 hours until it became a clear yellowish colour solution. This volume was reduced to 5ml and treated with methanol. The resulting brown crystals were collected and washed well with ethanol and acetone. The analytical data are given in Table 3.3.

(ii) Preparation of the Coordination Compound [Pt(2-Amino-4-methyl BTZ)$_2$Cl$_2$] :

A mixture of $PtCl_2$ (500mg) and ligand 2-amino-4-methyl benzothiazole (1gm) in water and methanol (50ml) was refluxed at 80^{0}C 5-6 hours until it became a clear white colour solution. This

volume was reduced to 5ml and treated with methanol. The resulting white crystals were collected and washed well with ethanol and acetone. The analytical data are given in Table 3.3.

The general reaction for the preparation of coordination compounds of platinum is as follows :

$$[Pt\ (Cl)_2] + 2L \xrightarrow[H_2O]{CH_3OH} [Pt\ (L)_2(Cl)_2]$$

where L = 2-Amino-6-methyl benzothiazole and 2-amino-4-methyl benzothiazole

(c) Analysis of the Constituents Elements :

(i) Carbon, Hydrogen, Nitrogen and Sulphur present in the investigated complexes were estimated micro-analytically.

(ii) Estimation of Pt :

For the estimation of platinum as ammonium chloroplatinate, dissolved the compound in 5ml of concentrated hydrochloric acid and 20ml of hot water, and then add gradually an equal bulk of half-saturated ammonium chloride solution. Allow to stand for 8 hours, filtered off the precipitate, wash it with ammonium chloride solution, and finally twice with cold water. Transfered the filtered paper and precipitate to a Main-Smith Crucible, heat extremely slowly at first, and ultimately raise to a bright red heat. Repeated heating, cooling and weighting were carried out until weight obtained constant.

(d) Physical Methods :

(i) Molecular Weight Determination :

Molecular weight determination of the synthesized complexes were made by Rast's Method.

(ii) Magnetic Susceptibility Measurement :

The magnetic susceptibility measuerments were made at room temperature by the Gouy Method. A magnetic field strength of 8500 guass was employed. The apparatus was calibrated using cobalt mercury thiocynate $Hg[Co(NCS)_4]$. The diamagnetic corrections were computed using Pascal's Constant **[45,46]**. For calculations of effective magnetic moment following equation has been used.

Effective magnetic moment (μ eff) = 2.84 $(X^{corr}m\ T)^{½}$, where T = temperature in absolute scale and X_m = corrected molar susceptibility.

(iii) Conductance Measurement :

Conductance was measured in analytical grade methanol using dip type cell with the help of a Philips Conductivity Bridge.

(iv) Infrared Spectral Measurement :

Infrared spectra (4000-600cm^{-1}) of the uncoordinated ligands and of the complexes were recorded as Nujol Mulls supported between sodium chloride platex (rock salt regions) on a Perkin Elmer Spectrum RXI Spectrometer.

(v) ^{1}HNMR Spectral Measurement :

^{1}HNMR spectra of the synthesized compounds will be recorded on AC 300F Spectrometer (300MH_z) using TMS as an internal standard.

(vi) Electron Spin Resonance Spectra :

Electron Spin resonance spectra of the complexes were recorded at room temperature on a Varium E-3 spectrometer using powdered sample at the microware frequency 9.53GHz. The 'g' values were calculated using the given equation.

$$g = \frac{714.44 \times \sqrt{(GHz)}}{H\ (G)}$$

where $\sqrt{}$ (GHz) = microwave frequency in GHz at which sample operated, and H (G) = field in Gauss for the sample.

3.3 : Properties of the Complexes :

The analytical and physical data of the ligand and its metal complexes are given in Table no 3.3. The complexes are non-hygroscopic and stable at room temperature. The solubility of these complexes are given in Table no 3.5. They are soluble in DMF and DMSO, Slightly soluble in chloroform and insoluble in other organic solvent. The colour of these complexes are given in Table no 3.4. They do not possess sharp melting points.

3.4 : Result and Discussion :

(a) Magnetic Measurement :

The magnetic values of synthesized complexes measured at room temperature. An observation shows that the magnetic moment values of the complexes [Pt(2-amino-4-methyl BTZ)$_2$Cl$_2$] and [Pt(2-amino-6-methyl BTZ)$_2$Cl$_2$] are zero. Hence, all the

complexes are diamagnetic and also the square-planar geometry of compounds are evident from their diamagnetic nature.

(b) Conductance Measurement :

The analytical and physical data of the ligand and its metal complexes are given in Table no 3.3. The values of molar conductance are in the range 0.052-0.058 Ω^{-1} cm^2 mol^{-1} suggesting non-electrolyte nature of the synthesized complexes.

(c) Infrared Spectroscopy :

The ligand 2-amino-6-methyl benzothiazole and 2-amino-4-methyl benzothiazole possess three possible donor sites; (i) amino nitrogen, (ii) tertiary cyclic nitrogen and sulfur of thiazole ring. Further the amino group involved in coordination through the nitrogen atom. Coordination through nitrogen of the amino group invariably results in the negative shift in υNH_2 (3105 cm^{-1}) by at least 60 cm^{-1} (3045 cm^{-1}) in 2-amino-6-methyl benzothiazole and 50 cm^{-1} in 2-amino-4-methyl benzothiazole (3055 cm^{-1}). In the complexes of 2-amino-6-methyl benzothiazole and 2-amino-4-methyl benzothiazole, the IR frequency of tertiary cyclic nitrogen and sulfur of thiazole ring are unchanged, suggesting cyclic nitrogen and sulfur of this ligand do not participate in the coordination.

(d) Electron Spin Resonance Spectra :

The electron spin resonance data for the synthesized complexes under this investigation are given in Table 3.7.

The recorded 'g' values in the range 1.982-1.988 are constant.

The electronic spectral bands of the complexes (Table 3.8) were assigned according to the literature **[47,48]**.

The molecular orbital approach was used to explain the structure of square-planar complexes of the d^8 elements. The metal orbitals involved in σ-bonding in square–planer complexes are the nd_{z^2}, $nd_{x^2-y^2}$ (n+l)s, (n+1)P_x and (n+1)$P_{y'}$. Nevertheless, judging from the values of the overlap integrals, ndx^2-y^2 (n=1)s, (n+1)P_x and (n+1)P_y account for most of the σ-bonds, and ndz^2 makes only a minor contribution. The most important π–molecular orbital and a combination of π–orbitals of the ligands.

The correlation of the bands observed in the electronic spectra for the studied complexes with those of $[M(CN)_4]^{2-}$ [M=pt^{11}] prompted us to assume the following assignments (Table 3.8) $^1A_{1g} \rightarrow {}^1A_{2g}[b_{2g}(\pi^*) \rightarrow b_{1g}(\sigma^*)]$, (d-d); $^1A_{1g} \rightarrow B_{1g}$ $[b_{2g}(\pi^*) \rightarrow a_{1g}(\sigma^*)]$, (d-d); $^1A_{1g} \rightarrow {}^1E_g[e_g(\pi^*) \rightarrow b_{1g}(\sigma^*)]$, (d-d); $^1A_{1g} \rightarrow {}^1B_{1u}$ $[b_{2g}(\pi^*) \rightarrow a_{2u}(\pi^*)]$, (C.T); $^1A_{1g} \rightarrow {}^1E_u[e_g(\pi^*) \rightarrow a_{2u}(\pi^*)]$, (C.T).

The relation between the bands in the present complexes and the described for the typical complexes $[M(CN)_4]^{2-}$ leads to the conclusion that all the new complexes have the same square-planar geometry.

(e) NMR Spectroscopy :

The spectrum of Pt (II) with 2-Amino-6-methyl benzothiazole and 2-Amino-4-methyl benzothiazole complexes dissolved in D_2O consists of a broad NH_2 signal at 5.38ppm. At later times, or raising signal the pH, the NH_2 protons exchange completely. Spectrum of aforesaid compounds shown into figure.

The methyl proton spectrum consists of doublet, due to N-H coupling, flanked by symmetric doublet ^{195}Pt side bands.

Effect of Coordination on Chemical Shifts :

Coordination to Pt invariably produces substantial downfield shifts for ligand protons. The extent of the downfield shift is similar to corresponding protonation shifts and decreases similarly with distance from the coordination site. Among the methyl-substituted glycines, chelation produces a downfield shift about 80% as great as that resulting from single protonation. Not surprisingly, CH_2 signals of N-coordinated glycinates are shifted downfield only half as much as those of chelated glycinates, but are much more affected by protonation of the free CO_2^- group.

Such observations are the basis for concluding that the α-carboxyl group, rather than the β-carboxyl group, is involved in chelation top Pt in complexes of L-aspartate. For those complexes, the methane proton is more affected by complexing, while the methylene protons are more affected by subsequent titration of the uncomplexed carboxyl. For S-methylcysteine, and methionine, the effect of protonation of NH_2 and CO_2^- on CH_3 shifts is not large, but coordination of NH_2 to Pt produces a downfield shift comparable to the effect of protonation or complexing N for $N-CH_3$ groups.

Differences in chemical shifts for all ligand protons were negligible for the following pairs of isomers: *trans*-Pt(L-Asp)$_2$ and Pt(L-Asp)(D-Asp), *trans*-Pt(L-Glu)$_2$ and Pt(L-Glu)(D-Glu), and *cis*- and *trans*-Pt (L-Dap)$_2$. For Pt(L-Hist)$_2$, chemical shifts of *cis* and

trans isomers are very similar for corresponding protons of corresponding species, except for C_2-H whose environment in the two isomers varies significantly.

Platinum-Proton Coupling and Coordination :

The presence or absence of Pt side bands provides a clear identification of the site of coordination in these complexes. In general, three-bond Pt-N-C-H couplings are between 10 and 60

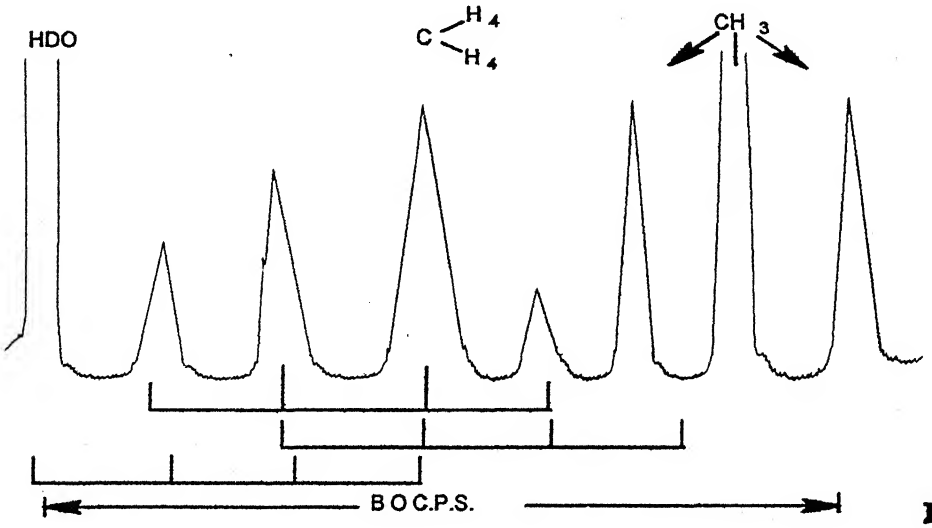


Fig. 3.1: Proton nmr spectrum of the sarcosine complex $K[Pt\text{–}(NDCH_3CH_2CO_2)Cl_2]$ in D_2O.

Hz. Coupling through four bonds is negligibly small, except for C_4-H of histidine which is coupled through four bonds nearly as strongly as is C_2-H through three bonds **[49]**.

Not surprisingly, in view of the known strong tendency of Pt(II) to coordinate sulfur, $J_{Pt\text{-}S\text{-}C\text{-}H}$ values are generally among the largest values observed. A small, but noteworthy, variation in $J_{Pt\text{-}CH3}$ was observed for 1 : 1 complexes of both S-methylcysteine and methionine. Starting with $Pt(AA)Cl_2$, replacement of the Cl *cis*

to the N of AA by NH_3 decreases $J_{Pt\text{-}CH_3}$ from ~55 to ~45 Hz, while replacement of both Cl's by NH_3 or ethylenediamine has a smaller effect (~55 to ~50 Hz).

3.5: Summary :

The mixed ligand complexes $[PtL_2Cl_2]$ where (L=2-amino-6-methylbenzothiazole and 2-amino-4-methylbenzothiazole), have been prepared by the interaction of parent compound $[PtCl_2]$ with ligand. The complexes are characterized by elemental analysis, magnetic measurement, electron spin resonance and infrared spectral studies containing Pt (II) d^8 configuration. All the complexes are diamagnetic suggesting square planner geometry. It is observed that:

(i) The DMF solution of the synthesized compounds are non-conducting.

(ii) All the complexes contain low spin d^8 configuration.

(iii) The reflectance spectra of the complexes display a shoulder at 340-430 nm, which is attributable to transition

$A_{1g} \longrightarrow A_{2g}$

(iv) All the compounds are thermally stable upto 200^0C.

(v) All the complexes show anticancer activity.

Table 3.1 :

Physiochemical Comparative Data for the Electrophilic Reactivity of Nitrogen of Pyridine and Thiazole

	Thiazole	**Pyridine**
pK_a	2.52	5.27
$\Delta H(BF_3)$[a]	123.93	139.32
K_2(MeI)[b]	15.8×10^{-6}	35.0×10^{-6}

[a]Enthalpy of reaction with BF_3 in $PhNO_2$ at 25°C, in J mol^{-1}.

[b]Specific rate of methylation by MeI in acetone at 40°C, in 1 mol^{-1} s^{-1}.

Table 3.2 :

pK_a of Some Representative Thiazoles [44]

Thiazole	pK_a	Thiazole	pK_a
Thiazole	2.52	4-Ethylthiazole	3.20
2-Methylthiazole	3.43	2-Ethylthiazole	3.37
4-Methylthiazole	3.15	4-t-Butylthiazole	3.06
5-Methylthiazole	3.12	2-t-Butylthiazole	3.15
2,4-Dimethylthiazole	3.98	2-Aminothiazole	5.39
2,5-Dimethylthiazole	3.91	Benzothiazole	1.2
4,5-Dimethylthiazole	3.983.73		

Table 3.3 :

Analytical and Electronic Spectral Data of Pt (II) Complexes

Compound	Found(Calc.)%						Mol.Wt.
	M	C	H	N	S	Cl	Found(Calc)
	32.42	32.00	2.235	9.06	10.23	11.42	593.97
$[Pt(C_8H_8N_2S)_2Cl_2]$	(32.82)	(32.33)	(2.71)	(9.43)	(10.79)	(11.93)	(594.54)
	32.37	32.00	2.26	9.00	10.26	11.33	593.94
$[Pt(C_8H_8N_2S)_2Cl_2]$	(32.82)	(32.33)	(2.71)	(9.43)	(10.79)	(11.93)	(594.54)

Table 3.4 :

Colour, and % Yield of the Complexes

S.No.	Compound	Colour	%Yield
1.	[Pt(2–amino–6–methyl BTZ)$_2$Cl$_2$]	Brown	68
2.	[Pt(2–amino–4–methyl BTZ)$_2$Cl$_2$]	White	57

Table 3.5 :

Solubilities of the Complexes in Different Solvents

S.No.	Compound	DMF	DMSO	EtOH	MeOH	Chloroform	Ethylacetate
1.	[Pt(2–amino–6–methyl BTZ)$_2$Cl$_2$]	Soluble	Soluble	Insoluble	Insoluble	Sparingly soluble	Insoluble
2.	[Pt(2–amino–4–methyl BTZ)$_2$Cl$_2$]	Soluble	Soluble	Insoluble	Insoluble	Sparingly soluble	Insoluble

Table 3.6 :

Important IR Spectral Bands and Their Assignments

S.No	Compound	υ (NH_2)	υ C-$S_{(cyclic)}$	υ $N_{(cyclic)}$
1.	[Pt(2-amino-6-methyl BTZ)$_2Cl_2$]	3045 cm^{-1}	unchanged (780-740cm^{-1})	unchanged (1375cm^{-1})
2.	[Pt(2-amino-4-methyl BTZ)$_2Cl_2$]	3055 cm^{-1}	unchanged (780-740cm^{-1})	unchanged (1375cm^{s})

Table 3.7 :

Electronic Spectral Data of the Complexes

$[Pt(L)Cl_2]$	21701	$^1A_{1g} \rightarrow {}^1A_{2g}[b_{2g}(\pi^*) \rightarrow b_{1g}(\sigma^*)]$
	265003	$^1A_{1g} \rightarrow {}^1B_{1g}\ [b_{2^*}(\pi^*) \rightarrow a_{1g}(\sigma^*)]$
	304002	$^1A_{1g} \rightarrow {}^1E_g[e_g(\pi^*) \rightarrow b_{1g}(\ \sigma^*)]$

Table 3.8 :

Electronic Spectra of MCl_2L_2 Chelates

Transition	**$[PtL_2Cl_2]$**
d-d	
$^1A_g \rightarrow {}^3B_{1g}(x_2\text{-}y_2 \rightarrow xy)$	
$^1A_{1g} \rightarrow {}^1B_{1g}(x_2\text{-}y_2 \rightarrow xy)$	18, 183 (3.48)
$^1A_g \rightarrow {}^3B_{3g}(xz \rightarrow xy)$	
$^1A_g \rightarrow {}^1B_{3g}(xz \rightarrow xy)$	17, 481 (3.55)
M→L charge transfer	
$^1A_{1g} \rightarrow {}^1B_{2u}[xz \rightarrow L(\pi^*)]$	17, 485 (3.55)
$^1A_g \rightarrow {}^1B_{3u}[yz \rightarrow L(\pi^*)]$	28, 003 (3.92)
M→L charge transfer	
$^1A_{1g} \rightarrow {}^1B_{2u}\ {}^1B_{3u}[L(\pi) \rightarrow xy]$	35, 712 (4.11)
$^1A_g \rightarrow {}^1B_{2u}\ {}^1B_{3u}[L(\sigma) \rightarrow xy]$	41, 844 (4.51)
L→L*	
$^1A_g \rightarrow {}^1B_{2u}$	29, 940 (4.01)
$^1A_g \rightarrow {}^1B_{1u}$	38, 170 (4.28)

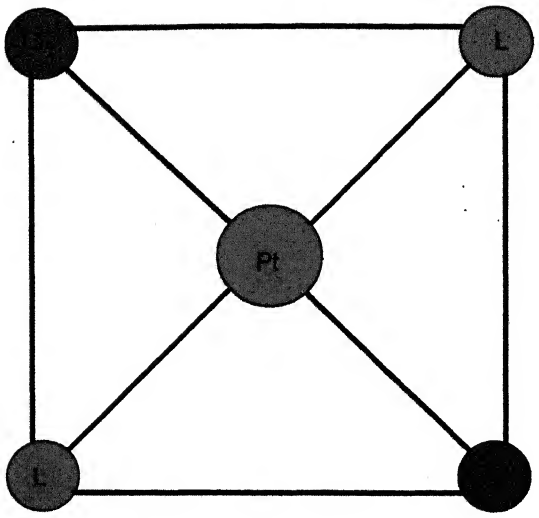
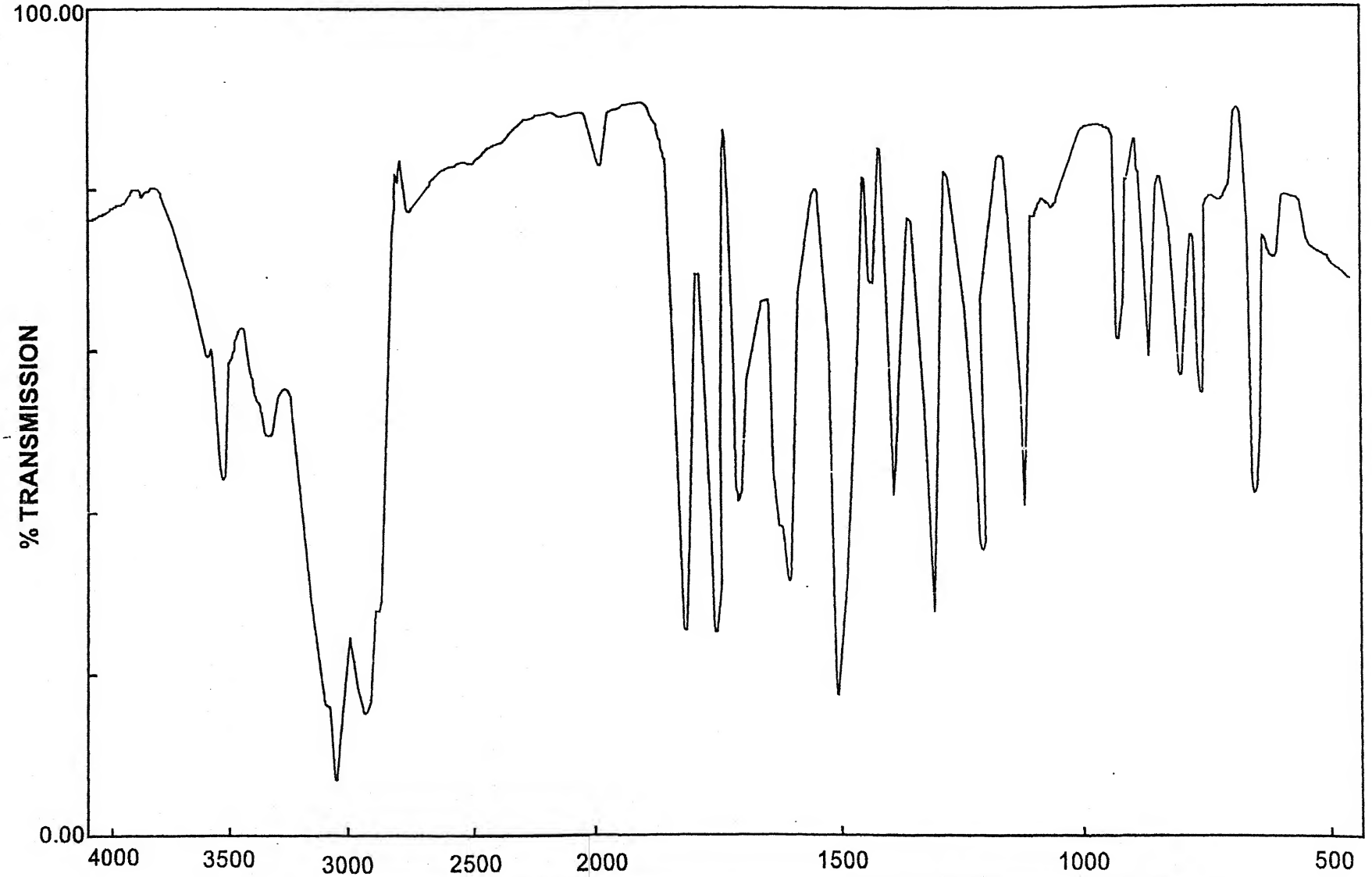


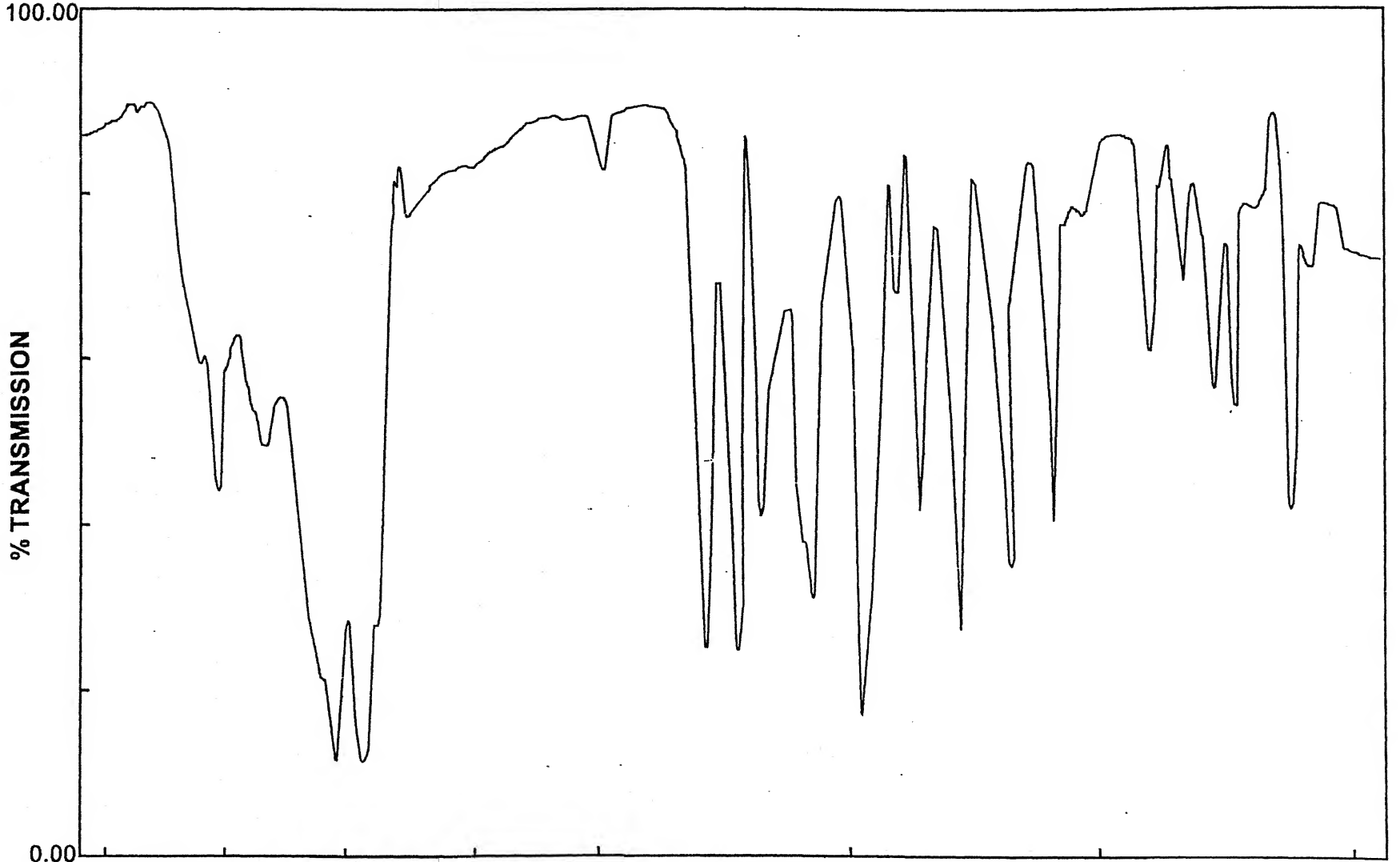
Fig .3.2 : Proposed square planar structure of $[Pt(L)_2Cl_2]$ (where L= 2-amino-6-methyl benzothiazole, 2-amino-4-methyl benzothiazole)

Fig 3.3 : 2- Amino 6-methyl beazothiazole

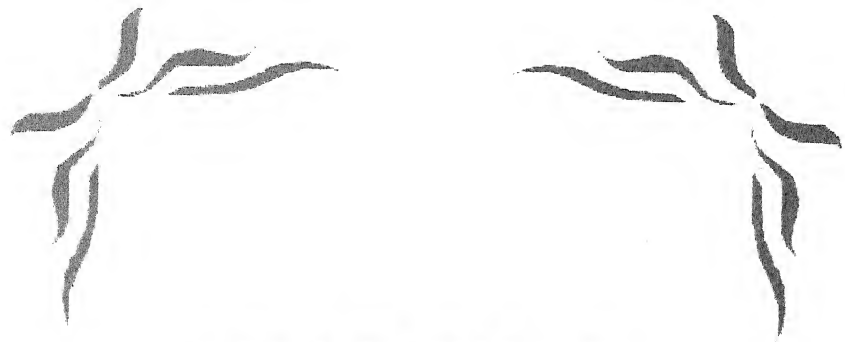
Fig 3.3 : 2- Amino 4-methyl beazothiazole



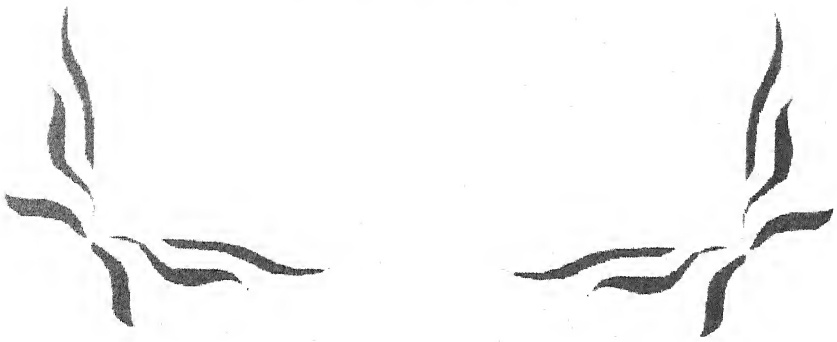
INFRARED SPECTRUM OF $[Pt(2\text{-Amino-6-Methyl BTZ})_2Cl_2]$



INFRARED SPECTRUM OF [Pt(2-Amino-4-Methyl BTZ)$_2$Cl$_2$]



CHAPTER-IV

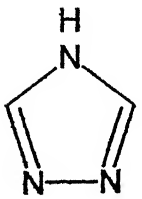


CHAPTER-IV

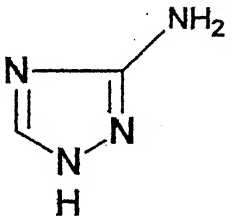
4.1 : Introduction :

In Chapter IV, as a part of our programme to synthesize and characterize some neutral-mixed ligand complexes of divalent platinum, studies have been extended using some triazole complexes.

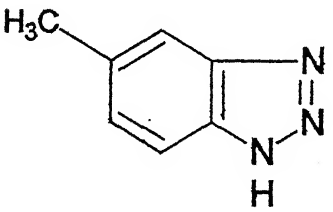
In recent years a great deal of interest have been shown in the study of mixed ligand platinum complexes having d^8 electronic configuration with 1,2,4-triazole, 3-amino-1,2,4-triazole, 5-methyl benzotriazole and 5-nitro benzotriazole.



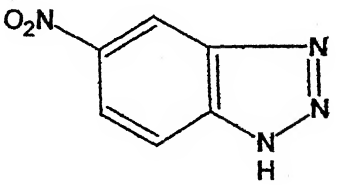
1,2,4-Triazole



3-Amino-1,2,4-Triazole



5-Methyl Benzotriazole

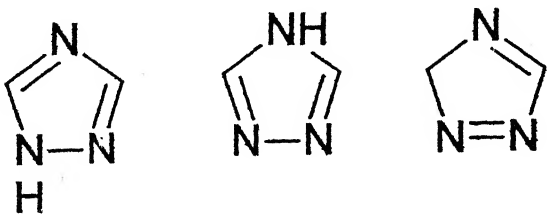


5-Nitro Benzotriazole

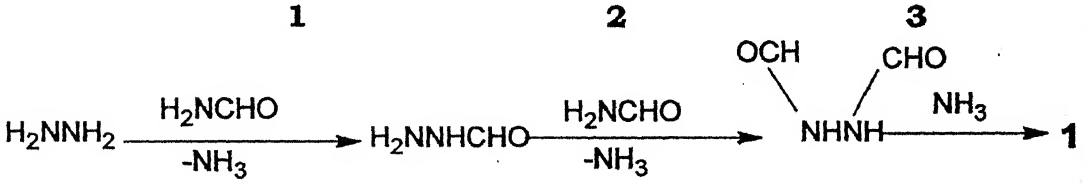
Bladin **[50c,d]** synthesized the first derivatives of 1,2,4-triazole and correctly represented their cyclic structure. The present chapter describes the result of such studies.

The heteroaromatic triazole ring system is composed of five atoms, two carbons, and the three nitrogen, which can be arranged in

two combinations to give either 1,2,3-triazole. Although two NH **(1and2)** and one CH_2 **(3)** tautomeric forms are possible for 1,2,4-triazole, this



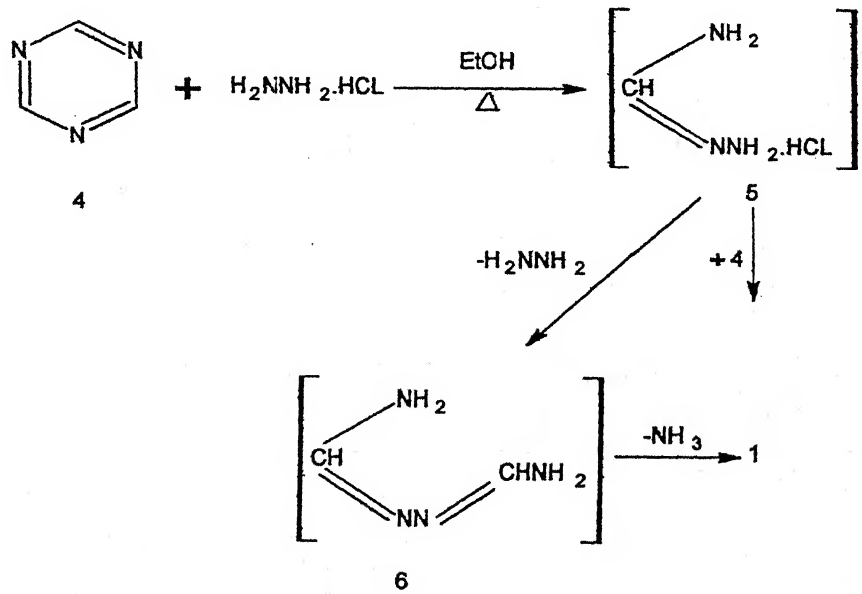
1 **2** **3**



structure is the best representation as a positively charged hydrogen associated with the resonance stabilized triazole anion **[51]**. *In Chemical Abstracts* 3-substituted and 3,5-disubstituted 1,2,4-triazole are usually indexed as s-triazoles. The 1,2,4-1H-triazoles notation is used to describe a 1*N*-substituted triazoles, whereas, 1,2,4-4H-triazoles is used to describe a 4*N*-substituted triazole. Trivial names such as guanazole(3,5-diamino-s-triazole), guanazine(3,4,5-triamino-1,2,4-4H-triazole), and either bicarbamimide or urazole(1,2,4-triazolidine-3,5-dione) have been replaced by systematic names throughout this chapter. In addition to reviews by Potts **[51]** and Boyer **[52]**, the relationship of the 1,2,4-triazoles in regard to other small-ring azoles has been reviewed recently by Schofield, Grimmett, and Keene **[53]**.

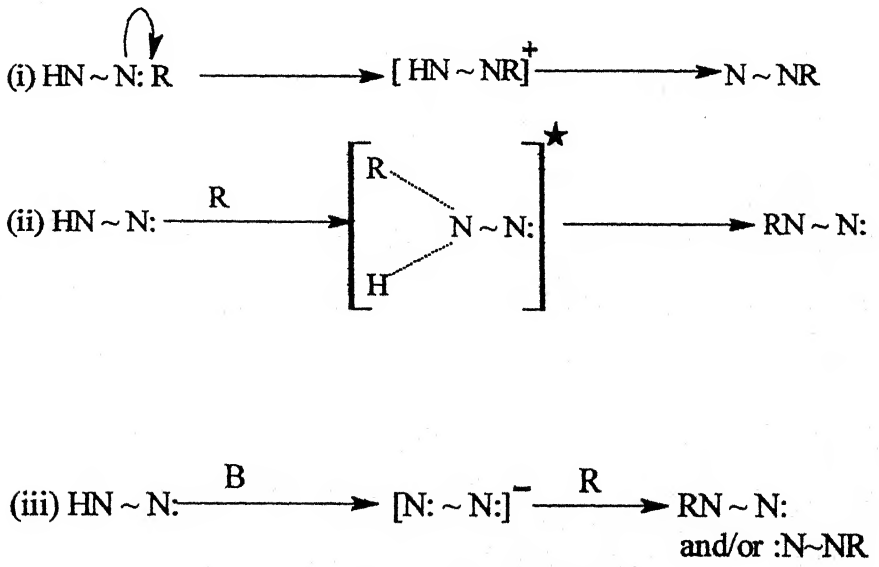
Bladin reported the preparation of derivatives s-triazole **(1)** in 1885 **[54]**, and soon, thereafter, Pellizzari obtained the parent ring system from the reaction of formylhydrazine with formamide **[55]**. This

and related reaction, which gave low and variable yields of s-triazole **(1)** have been reviewed **[51]**. Later the condensation of hydrazine sulphate with formamide was reported to give a 53% yield of s-triazole **(1)** **[56]**. Ainsworth and Jones observed that a large quantity of ammonia was evolved in the reaction of hydrazine with formamide, and to prevent the loss of ammonia, the intermediate *N,N'*-diformylhydrazine was reacted with excess ammonia in a pressure vessel to give a 70 to 80% yield of s-triazole **(1)** **[57]**. A further improvement in the yield of s-triazole **(1)** resulted from the work of Grundmann and Ratz, who obtained a 95% yield of s-triazole **(1)** from the interaction of s-triazine **(4)** with hydrazine hydrochloride **[58]**. Apparently, the intermediate amidrazone **(5)** was initially formed, which was postulated to react with another molecule of s-triazine **(4)** to give s-triazole **(1)**. However, the acid-catalysed self-condensation of amidrazone is well documented, **[59-61]** and s-triazole **(1)** might be formed via intermediate **(6)**. With hydrazine rather than its hydrochloride, s-triazine **(4)** reacted to give 1,2-diformylhydrazine dihydrazone **[62]**.



(a) Reactions at the Nitrogen Atoms :

The presence of two or more nitrogen atoms in the azoles multiplies the number of ways in which an *N*-unsubstituted compound can be converted into an *N*-substituted one. The possibilities, recalling those existing with pyridine derivatives containing tautomerisable substituents are illustrated here. The first process is that of quaternisation of the pyridinic nitrogen atoms followed by, or accompanying (S_E2') proton loss. With compounds in which the imino-hydrogen atom is already replaced, say by an alkyl group, quaternisation will result. The second process is the S_E2 reaction, and should be related to the energy needed to localize two electrons on the nitrogen atom being attacked. The third process is that of electrophilic attack on the conjugate base of the parent heterocycle (SE2cB) **[63]**.

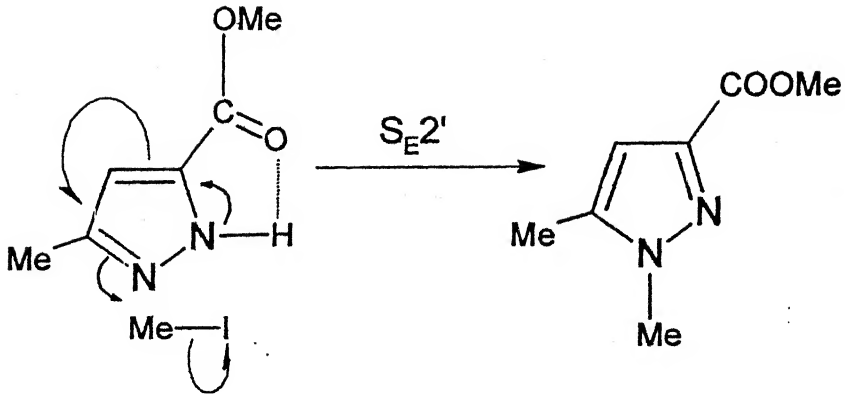


Whilst these possibilities are clearly present, a priori, there is, in fact, except in the case of the alkylation of imidazoles, very

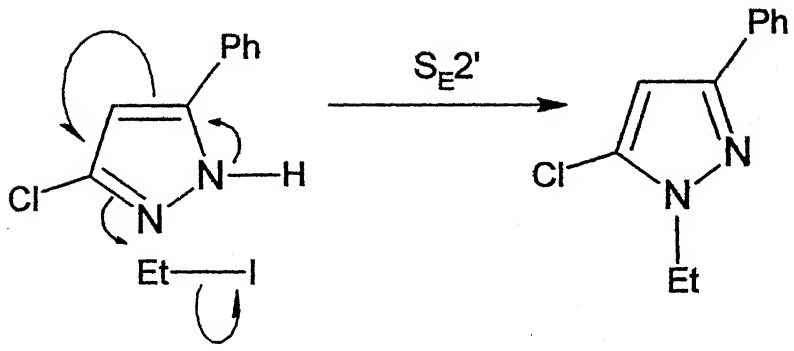
little information about the mechanisms concerned in most of the *N*-substitutions to be mentioned. Sometimes the experimental conditions permit reasonable guesses; this, reactions carried out with metal salt of azoles seem likely to be of the SE2cB type .

(i) The Attachment of Alkyl and Substituted Alkyl Groups: Substitutive Alkylation :

Pyrazoles have been *N*-alkylated by being heated with alkyl halides **[64a,b, 65a-g, 66b,g,i]**, or substituted alkyl halides such as methyl bromoacetate **[67]** or ethylene chlorhydrin **[68]**. The mechanisms of these reactions are not known and might be SE2 or S_E2'. In these reactions there are formed salts from the halogen hydracids liberated, and also small proportions of quaternary salts from the *N*-alkylpyrazoles and the alkyl halides. An unsymmetrical pyrazole usually gives both possible *N*-alkylated isomers **[65b,e,f,67]**. Thus, 3-phenylpyrazole with methyl bromide at 100^0C gives a mixture of 1-methyl-3-phenyl- and 1-methyl-5-phenyl-pyrazole **[65b]**. On the other hand, 3-chloro-5-phenylpyrazole with ethyl iodide gives only 5-chloro-1-ethyl-3-phenylpyrazole **[66b]**. 3-Methyl-pyrazole with ethyl bromide or methyl iodide gives comparable amounts of the possible isomers **[65f]**, whilst 3-phenylpyrazole and ethyl bromide provide mainly 1-ethyl-3-phenyl-, and only a little 1-ethyl-5-phenylpyrazole **[65g]**. Methyl 3-methylpyrazole-5- carboxylate gives methyl 1,5-dimethylpyrazole-3-carboxylate with methyl iodide **[66i]**. This last case, and that of 3-chloro-5-phenylpyrazole are interesting. It might be argued that in the ester the tautomer



(4.1)



(4.2)

(4.1) will be the dominant form, whilst with the chloro-compound the base-weakening influence of the halogen upon the adjacent nitrogen atom will render (4.2) the major form. In S_E2' reactions (4.1) and (4.2) would provide the isomers actually isolated.

As suggested above, it is likely that alkylations in which pyrazole salts are used are of the S_E2cB type. Silver pyrazole and methyl iodide at 120^0C give 1-methylpyrazole **[69f,70a,71g],** and the salt reacts similarly with β-chloroalanine **[72]**. Pyrazole **[73b]**, 3-methyl-, and 3,5-dimethyl-pyrazole have been *N*-alkylated with alkyl halides and alcoholic sodium alkoxides **[65a]**.

An unsymmetrical pyrazole can give two isomers when *N*-alkylated by these methods, but the proportion of each formed depends on the conditions and the nature of the substituents present **[74b,c,64a,65b,d,e,f,g,j,66a, b,d,i]**. It is most probable that in a number of cases where the formation of one product is reported modern techniques of analysis might reveal the presence of the second isomer, and where two isomers are reported to be formed might not confirm the reported proportions of each one. A study of this problem is desirable, and in the meantime the following report can only be accepted with caution. Thus, sodium 3-chloro-5-methylpyrazole with methyl iodide in absolute ether is said to give 3-chloro-1,5-dimethylpyrazole, whilst in moist ether a little of the isomer is also formed. Silver 3-chloro-5-methylpyrazole with methyl iodide gives equal amounts of the two isomers, and the use of dimethyl sulphate and alkali produces both 3-chloro-1,5- and 5-chloro-1,3-dimethylpyrazole, the fomer predominating **[74b,66a,i]**. 3-Methylpyrazole with methyl iodide and sodium methoxide gives about equal parts of 1,3- and 1,5-dimethylpyrazole; ethyl bromide under the same conditions gives a similar result, but from benzyl chloride the main product is 1-benzyl-3-methylpyrazole **[65f]**. 3-Nitropyrazole with aqueous ethanolic potash and methyl iodide gives 1-methyl-3-(74%) and 1-methyl-5-nitro-pyrazole (26%) **[75]**.

3-Chloro-5-methylpyrazole with ethanolic sodium ethoxide and ω-halogeno-alkanoic esters gives products formulated as the 1-substituted compounds, but the orientations are not

established **[76e]**. With the same base and benzyl chloride, ethyl pyrazole-3-carboxylate is also believed to give the 1-benzyl compound, as is 3-phenylpyrazole with ethyl bromoacetate, with which pyrazole also reacts **[73d]**. Under these conditions ethyl pyrazole-3,4-dicarboxylate and methyl iodide give both possible isomers **[77]**. Sodamide has also been used as the base in the *N*-alkylation of pyrazoles **[78b]**.

The use of aqueous alkali and dimethyl sulphate has already been instanced above, and these reagents are probably the most used for the purpose of *N*-methylating pyrazoles. They have been used with 4-nitro- **[68]** and 3,5-dimethyl-4-nitro-pyrazole **[79b]**. With 4-iodo-3-methyl-pyrazole they give 4-iodo-1,3-dimethylpyrazole **[80]**.

Several pyrazoles have been *N*-alkylated by being heated with an alkyl halide and potassium carbonate **[81d]**. In this reaction with ethyl bromoacetate, toluene or 2-ethoxyethanol have been used as solvents **[80]**.

Potassium pyazole with chloroform in benzene gives tri-1-pyrazolyl-methane **[82a]**. The sodium salt of 3,5-dimethylpyrazole (prepared with sodium in toluene) has been normally *N*-alkylated **[83]**.

3,5-Dimethylpyrazole is substituted on nitrogen by the $PhCO.(CH_2)_2$ group by being heated with $PhCO.(CH_2)_2N\ Me_2$ **[83]**.

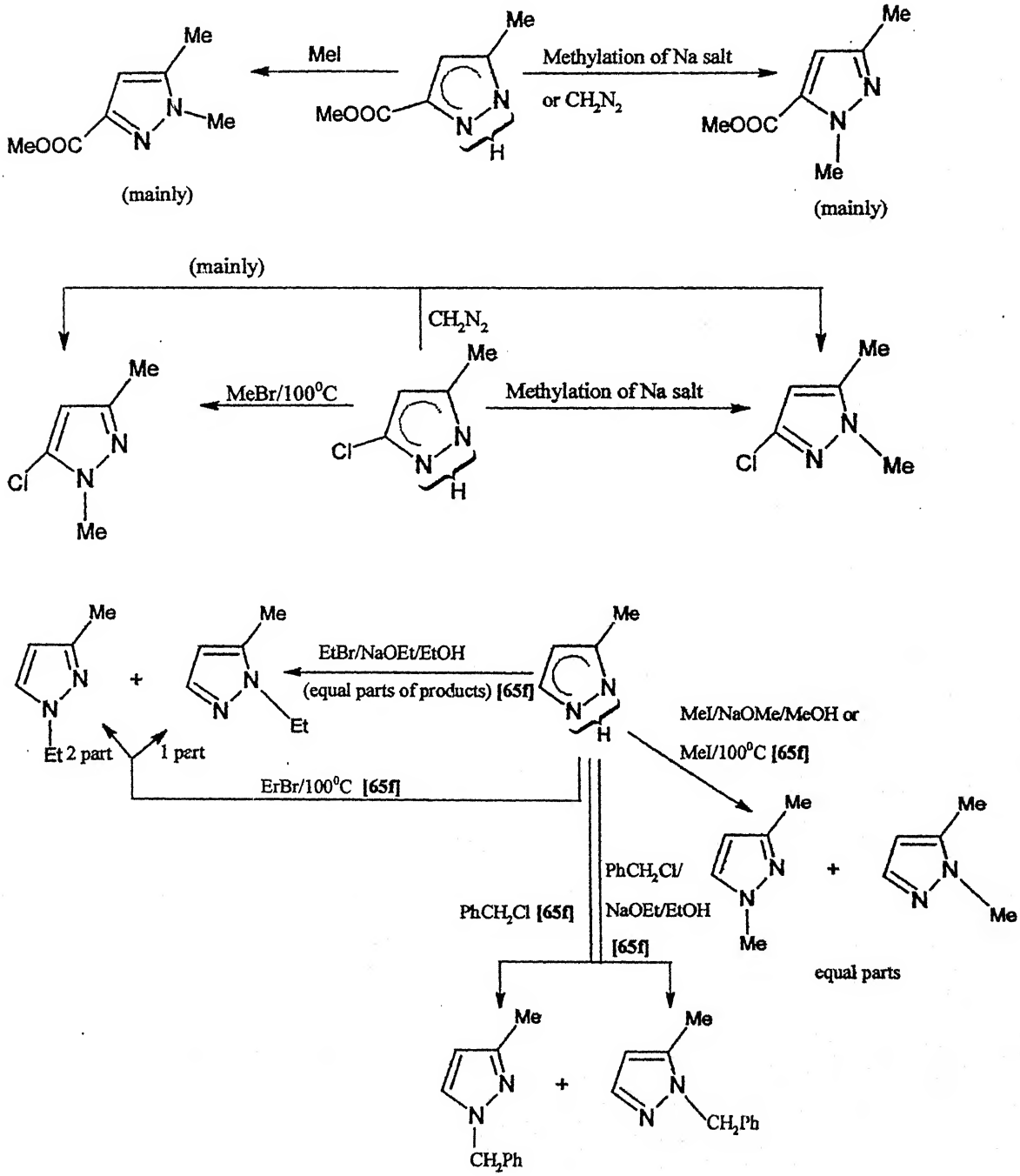
Diazomethane in ether has sometimes been used to *N*-alkylate pyrazoles, e.g. 3-chloro-5-methylpyrazole, 3-

methoxycarbonyl-4-phenyl- and 3-methoxycarbonyl-5-phenyl-pyrazole. The main products from the last two are the isomers *N*-methylated adjacent to the ester grouping **[65j]**, but the proportions of isomers, e.g. from 3-methoxycarbonylpyrazole vary with the conditions **[84a,b]**. The product from 3-chloro-5-methyl-4-nitropyrazole is probably a mixuture **[85a]**.

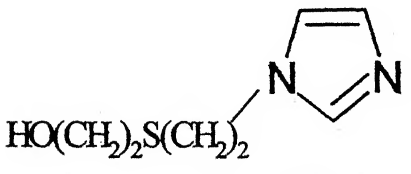
Methylations with diazomethane, like those with some other reagents, might when they produce mixtures of isomers be taken to indicate the occurrence of tautomerism in *N*-unsubstituted pyrazoles. Whether they do so or not depends on the mechanisms of the reactions. An older theory of the action of diazomethane suggested that this reagent placed a methyl group upon the atom, in this case nitrogen, which held the proton. Another possibility is that the diazomethane abstracts the proton and the resulting methyldiazonium cation then reacts with the mesomeric pyrazolyl anion. In this case the formation of two products merely reveals this mesomerism, and even if the older mechanisms applies the result demonstrates the existence of tautomerism without providing any evidence about the position of the equilibrium involved **[86d, 87]**. For the pyrazoles the mechanism is not known.

Some of the differing consequences of using various reagents or conditions from among the examples quoted above are illustrated above. As has been stressed, these reports must be accepted with caution.

Reaction of an imidazole with one equivalent of an alkyl halide **[88b,89b,90d,e,91d,e,65h,70a,92b,c,93-4,95]**or sulphate **[90d,96b, 97-9,95,100-o1]** effects *N*-alkylation. The former



reaction has been carried out neat, or in ether, alcohol, or benzene, the latter without a solvent or in water. The use of ether as solvent **[51e]** is disadvantageous **[102]**, and in all of these reactions a degree of quaternisation can occur. With mustard gas in dilute aqueous solutions at pH = 8.5 imidazole gives (4.3) and a quaternary picrylsulphonate supposedly of the cation (4.4) **[101]**. The structure (4.4) is not established and is highly improbable. As with the pyrazoles, an unsymmertrical imidazole generally provides both possible *N*-alkyl derivatives **[90d,96b,98-9,100]**. This is not always the case; 4-nitroimidazole with methoxymethyl chloride gave 1-methoxymethyl-4-nitroimidazole and other halides gave the analogous products, but yields were not high (21-54%). These products were orientated by n.m.r. spectroscopy **[97]**. 1,2,4-Trimethyl-5-nitroimidazole was obtained (64%) from 2,4-dimethyl-5-nitroimidazole and dimethyl sulphate at 100°C **[97]**, and in the same way 4-nitro-5-(*p*-acetamidophenyl) imidazole gave 1-methyl-5-nitro-4-(*p*-acetamidophenyl) imidazole **[103b]**. In quantitative studies imidazole was *N*-ethylated by reaction with ethyl methane sulphonate.



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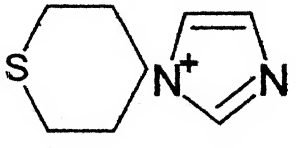
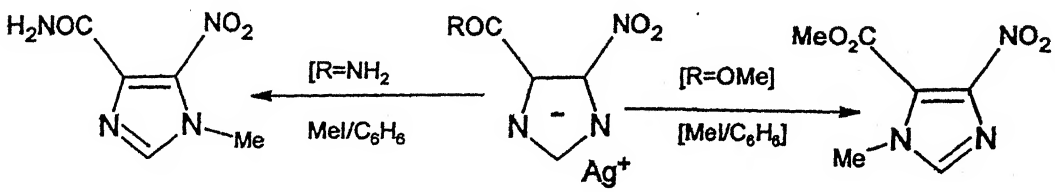


(4.4)

An important reaction of the present kind is that in which fusion of an acylated sugar with an imidazole in the presence of a

small proportion of chloroacetic acid gives an imidazole nucleoside. The method has been applied to the reaction of tetra-*O*-acetyl-β-D- ribofuranose with 2-nitroimidazole and 4-bromo-5-nitroimidazole. The latter gave only the 1-ribofuranosyl-5-bromo-4-nitroimidazole. These reactions are belived to be S_E2', and to occur through a carbonium or acetoxonium ion **[104a,b]**.

Imidazoles have also been *N*-alkylated by reaction of their silver salts with alkyl halides. Examples are the reaction of silver imidazole with acetyl bromide in xylene **[105]**, and of silver 4-nitroimidazole with methyl iodide in benzene which gives only 1-methyl-4-nitroimidazole **[106]**. Two other examples are illustrated here **[106-7]**.



Alkylation of silver salts is also used in the formation of imidazole nucleosides from acylglucosyl halides, the first example being the preparation of 1-glucosopyranosyl-5-methylimidazole (perhaps with some of the 3-isomer) from silver 4-methylimidazole and ά-acetobromoglucose **[108]**. The reaction has been used frequently **[104a,107-10]**, and proceeds with Walden inversion. Sometimes both possible isomers are formed. The chloromercuric salts are also useful, sometimes reacting when the silver salts fail **[104a]**.

The reactions of the silver salts of several imidazoles with triphenylmethyl chloride have been reported. Silver imidazole and 4,5-diphenylimidazole give the *N*-triphenylmethyl compounds, and that from the diphenyl compound rearranges to 4,5-diphenyl-2-triphenylmethylimidzole when heated **[111a]**. Other 4,5-diarylimidazoles behave similarly, the *N*-triphenylmethyl compounds giving 2-triphenylmethyl compounds when heated, though in some cases the original imidazole and decomposition products are formed **[111b]**. Silver 2,4,5-trisubstituted imidazoles do not react with triphenylmethyl chloride, but silver 4-phenyl- and 2,4-diphenyl-imidazole give *N*-triphenylmethyl compounds. Silver 2-phenylimidazole gave 2-phenyl-1-triphenylmethyl imidazole, which isomerised to 2-phenyl-4-triphenylmethyl imidazole when melted. Silver 2-t-butylimidazole gave 2-t-butyl-4-triphenylmethylimidazole **[111c]**.

Potassium imidazole gives 1-alkylimidazole by reaction with alkyl halides in various solvents at their boiling points or in sealed tubes **[112]**.

Imidazoles have been methylated with methyl iodide in sodium methoxide **[113]** or ethoxide **[103a]** solutions, and with sodium methyl sulphate and sodium ethoxide **[114]**. Since 4-(3,4-dichlorophenyl) imidazole gave 1-methyl-4-(3,4-dichlorophenyl) imidazole with methyl iodide in ethanolic sodium ethoxide, the products so obtained from several 4-aryl-imidazoles have been assumed to possess this orientation **[103a]**. The use of aqueous

methanolic sodium hydroxide with methyl iodide or butyl bromide gave good yields of 1-alkylimidazoles **[102]**.

Dimethyl sulphate and caustic soda have been used to alkylate imidazoles **[906a,b,d,96b],** and also methyl iodide or other alkyl halides with potassium carbonate and acetone. In contrast to methyl sulphate alone the latter reagents convert 4-aryl-5-nitroimidazoles into a mixture of both possible *N*-alkyl compounds **[103b]**, and with 2,4-dimethyl-5-nitroimidazole they give 1,2,5-trimethyl-4-nitroimidazole **[97]**.

Finally, diazomethane has been used to *N*-methylate imidazoles **[96b,113]**. The dominant formatioin of 5-substituted 1-methylimidazoles when the eventual 5-substituent is a group with a high electron density has been interpreted to indicate initial formation of an ion pair [$Im^{-}CH_3N_2^{+}$] in which the cation is situated near to the nitrogen atom next to the substituent **[86d]**.

Some details of the results of methylating imidazoles with various reagents are collected in Table 4.1. It should be noticed that yields from these reactions were generally not quantitative.

The work of Ridd **[63]** has provided a sound basis for the interpretation of these results. The reaction of 4(5)-nitroimidazole with dimethyl sulphate in dilute aqueous sodium hydroxide containing 10% of ethanol is homogeneous, and proceeds at a convenient rate at 25^{0}C. In these circumstances the nitroimidazole is present almost completely as the anion and the mechanism should be S_E2cB. From the observed kinetic form,

Rate = $k_2[Me_2SO_4],[Im]$,

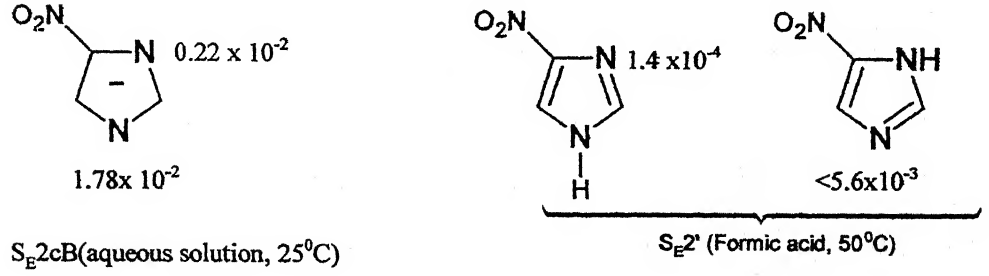
and isomer ratio (about 11% of the product is 1-methyl-5-nitroimidazole, and the rest is the isomer; this ratio for homogeneous methylation differs appreciably from that reported earlier (Table 4.1) the rate coefficient for each nitrogen atom was calculated. Anhydrous formic acid containing sodium formate was a convenient medium for studying the reaction in acidic circumstances, and the change in rate with sodium formate concentration led to the conclusion that

Rate= k_2'[Imidazole] $_{molecular}$[Me_2SO_4],

i.e. the mechanism was S_E2'. At least 86% of 1-methyl-5-nitroimidazole was formed, and no trace of the isomer was obtained. The kinetic results are expressed in the diagrams. In both cases the ratios of the nucleophilic activites of the two

Rate coefficients (1 mol^{-1} S^{-1}) for methylation of

4(5)-nitroimidazole with methyl sulphate



nitrogen atoms are much smaller than the ratio of their basicites. If a linear free-energy ralationship exists so that $K_{rate} \propto K^{-a}{}_{equilib}$; then the data are consistent with a~0.3. Such a relationship may be general. In imidazoles with [-*I*] substituents (R) at C-4(5), the predominant tautomer should be 4-R-imidazole, and methylation

in an S_E2cB process should give mainly 1-methyl-4-R-imidazole. With [+*I*] derivatives the situation should be reversed. With 4(5)-nitro-imidazole the conjugate base reacts about 10^3 times faster than the neutral molecules. Accordingly, the transition from S_E2cB to S_E2' should occur at about pH = pK_{acid}-3, that is, at about pH 6-11 for negatively substituted imidazoles. When 0 <a <1 the change from S_E2cB to S_E2' should change the main product of methylation.

Examination of the data summarized in Table 4.1 in the light of these considerations, shows that the preparative (heterogeneous) conditions give qualitatively the results to be expected. The discrepancies observed with halogeno-compounds in alkali and with 4(5)-phenylimidazole may be due, respectively, to the occurrence of S_E2' substitution of the imidazole dissolved in methyl sulphate and to steric factors.

The rates of reaction of some imidazoles with ethyl methanesulphonate in water at 35°C have been reported **[115]**. Imidazole, 2-methyl-, 4-methyl-, and2,4,5-trimethylimidazole give second order rate constants linearly related to p*K*a: evidently steric effects are almost absent in these reactions. Although 'a constant concentration of sodium hydroxide' was added 'to maintain the amine in the reactive form', these were evidently S_E2' reactions.

1,2,3-Triazole has been alkylated by reaction with propyl bromide, allyl bromide, ethyl bromoacetate, 2-chloropropionitrile, and β-phthalimidoethyl bromide in the presence of sodium

ethoxide. In each case the 1-alkyl compound predominated, ratios of 1-alkyl-1*H*-1,2,3-triazole to 2-alkyl-2*H*-1,2,3-triazole varying between 4 : 1 and 3 : 2. Use of the silver salt did not much change the ratios, whilst reaction with excess triazole alone gave almost exclusively the 1-alkyl compounds. The products were recognized by n.m.r. spectroscopy **[116]**. Similarly, methylation of 1,2,3,-triazole with caustic soda and dimethyl sulphate gave both isomers, but 1-methyl-1*H*-1,2,3-triazole predominated **[117c]**. Under these conditions 4,5-dibromo-1,2,3-triazole gave equal proportions of both isomers, but yields were not quantitative **[118]**.

Diazomethane produces roughly equal proportions of 1-methyl-1*H*- and 2-methyl-2*H*-1,2,3-triazole from 1,2,3-triazole **[119b]**, but 3-benzoyloxy-4-methyl- and 3-benzoyloxy-1,2,3-triazole give the 2-methyl compounds **[120e]**.

Most alkylations of 1,2,4-triazoles have been effected with a sodium alkoxide as the base. In this way methylation, ethylation, and allylation give the 1-substituted 1,2,4-triazoles. These and other results are collected in Table 4.2. It will be seen that 4-alkylation has been postulated to occur to a very small degree in one case, and that in two instances where it has been held to produce the sole product, the structures of these products have not been proved.

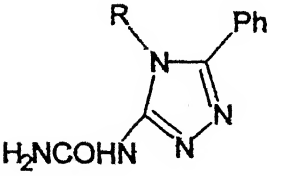
The situation existing in the pyridine series when alkylation of compounds containing tautomerisable substituents is considered, is complicated in the azoles by the possibility in

the latter of substitutive alkylation occurring at a nuclear nitrogen atom and also by the greater likelihood of *C*-alkylation. The case of quaternising alkylation is more properly compared with the reactions of the pyridines.

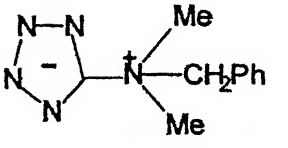
Little or nothing is known about the substitutive alkylation of aminopyrazoles. The cases of some aminopyrazolones are discussed below.

With dimethyl sulphate and alkali 4-amino-5-aminocarbonyl-1,2,3-triazole gave equal parts of both isomers, whilst the formyl derivative of this amine gave 4-aminocarbonyl-5-formamido-2-methyl-2*H*-1,2,3-triazole **[121c]**.

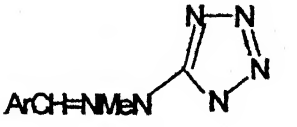
Benzyl chloride and caustic soda, and dimethyl sulphate and caustic soda, alkylate 3-phenyl-5-ureido-1,2,4-triazole at a nuclear nitrogen atom, but the reasons for preferring the structures (4.5), R= Me or $PhCH_2$, over the other possibilities are not convincing **[122c, 123]**.



(4.5)



(4.6)



(4.7)

The alkylation of 5-aminotetrazole has been studied in some detail. With dimethyl sulphate in water the sodium salt gives 5-amino-1-methyl-1*H*-1,2,3,4- and 5-amino-2-methyl-2*H*-

1,2,3,4-tetrazole, the former predominating **[124e,125,126]**. The total yield of these compounds was high, and the products of further methylation (1-and 2-methyl-5-methylamino tetrazole and 5-imino-1,3- and -1,4-dimethyltetrazole) were isolated in very low yields ($^{>}$ 1%). Methyl and ethyl iodide, allyl bromide, benzyl chloride, chlorohydrin, and ethyl sulphate similarly gave mixtures of 1- and 2-alkyl-5-aminotetrazoles in which the former predominated **[127,128c]**, as did diazomethane **[125]**. The particular case of benzylation and substituted-benzylation has been examined several times. The 'α-monobenzyl' compound formed with benzyl chloride in the presence of caustic soda **[129c]** was probably 5-amino-1-benzyl-1*H*-1,2,3,4-tetrazole **[130a,131a]**. Experiments with benzyl chloride and bromide and some *p*-substituted compounds showed reaction to occur at the amino group to the extent of about 10% of the proportion of nuclear attack. The proportions of the 1- and 2-substituted compounds formed were very similar and did not vary much **[132d]**.

As well as the 1- and 2-benzyl compounds this method also produces the ylide (4.6) **[133]** from 5-dimethylaminotetrazole.

5-Aminotetrazole gives both the 1- and 2-ethoxycarbonyl -methyl compounds with ethyl bromoacetate in the presence of triethylamine **[134]**.

Acting in the absence of alkali, alkylating reagents effect both substitutive and quaternising alkylation. These

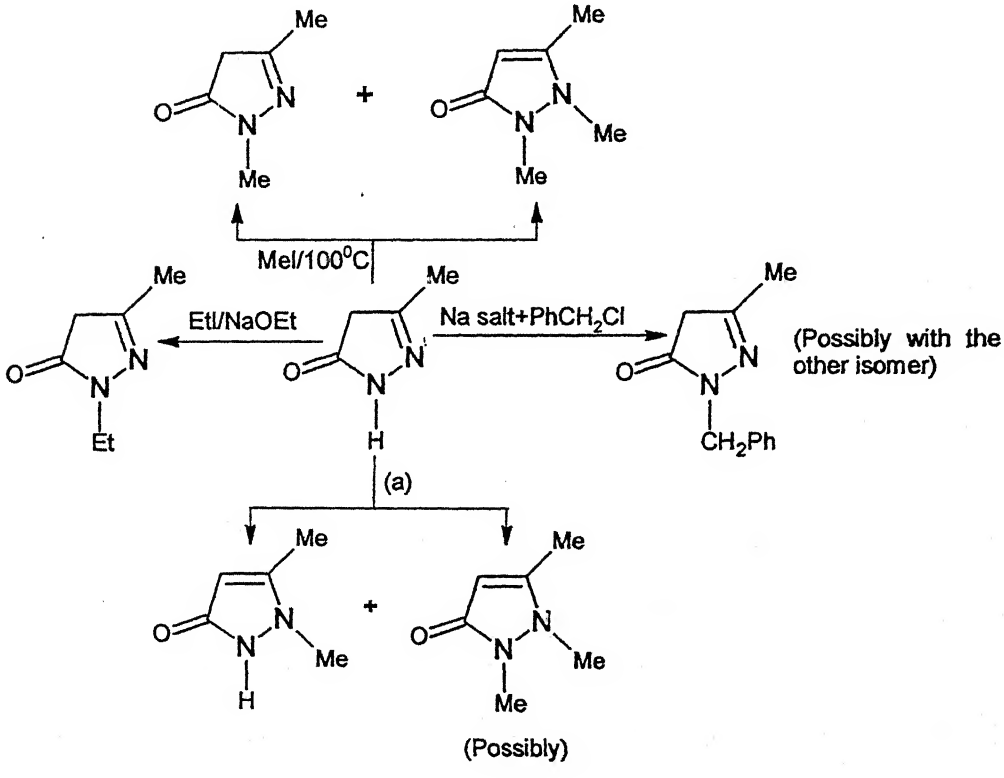
reactions are considered below, with the quaternisation of alkyl-5-aminotetrazoles.

In the related methylation of arylhydrazones of 5-hydrazinotetrazole, the main product is the 1-methyl compound, with some of the dimethyl compound (4.7), and a small amount of the compound monomethylated in the side chain. Increasing the proportion of alkali present increases the proportion of dimethylation, and with some hydrazones, the hydrazones of 5-hydrazino-2-methyl-2*H*-1,2,3,4-tetrazole were also formed **[135a,b]**. N.m.r. spectroscopy can be used to orientate the methyl derivatives of 5-aminotetrazole, but coincidence of signals prevents this with the methyltetrazol-5-ylhydrazones **[135c]**.

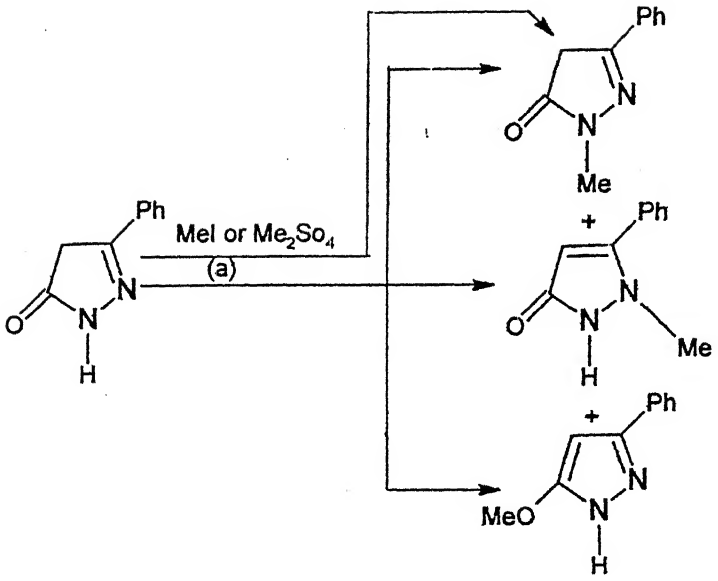
The alkylation of pyrazolones has received considerable attention, particularly in connection with the preparation of antipyrine and its analogues. For convenience these reactions will be divided into two sections, namely those starting from *N*-unsubstituted compounds, and those starting from *N*-substituted compounds.

The first reported alkylation of 3-methylpyrazol-5-one **[136e]** was unusual. By analogy with hydrazones it was expected that this compound would be reduced by being heated with sodium methoxide. In fact, ring opening to the extent of 41%, giving nitrogen and butyric acid, occurred, together with *C*-alkylation to produce 3,4-dimethylpyrazol-5-one (39%). 4-Ethylation and–propylation were similarly effected. These reactions recall the similar alkylations of pyrroles and phenols.

Other alkylations of 3-methylpyrazol-5-one **[74b,66a]** are summarized in the diagram. The general formation of 1-alkyl-3-methylpyrazol-5-ones by reaction with alkyl halides alone has been confirmed **[78a]**. From these reactions in the absence of base, the products are, of course, hydrogen halide salts, from which the alkyl derivatives are obtained by neutralization. A slightly different situation is found with 3-phenylpyrazol-5-one



(a) NaOMe/MeOH/MeI or $C_7H_7SO_3Me$



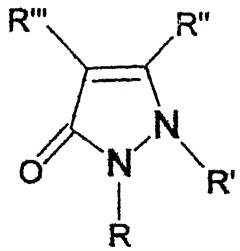
(a) Ref. **[66b]** says NaOMe/MeOH/MeI,
the experimental section NaOMe/MeOH/ $C_7H_7SO_3Me$

[66b], as shown, 3-Methyl-4-phenylpyrazol-5-one heated with methyl iodide gives 1,2,3-trimethyl-4-phenylpyrazol-5-one **[78a]**.

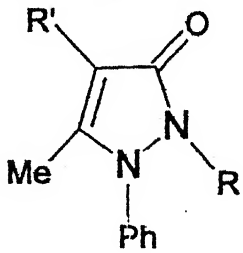
3-Pyrazolone reacts with xanthydrol, but it is not clear whether the product is a 1,2- or a 1,4-disubstituted compound **[137]**.

In 1884 Knorr discovered antipyrine (4.8), R = Ph, $R^{/}$ = $R^{//}$ = Me, $R^{///}$ = H, which he prepared by heating 3-methyl-1-phenylpyrazol-5-one with methyl iodide in methanol in a sealed tube at 100°C **[71a,b,138a]**. He later **[71k]** prepared antipyrine by ring-synthesis, and several analogues by similarly alkylating other 3-substituted 1-arylpyrazol-5-ones **[71a,c,k,l]**. Since Knorr's original observations, numerous analogues of antipyrine have been prepared by such alkylations **[139g,140b,138d,141b,78a,b,131b,142a]**, and derivatives of 3-antipyrine (4.9) have been similarly prepared **[143c]**.

Many variations of reagent and conditions for preparing antipyrine itself by methylating 3-methyl-1-phenylpyrazol-5-one have also been reported **[139h,144a,c,138c,145a-c]**.

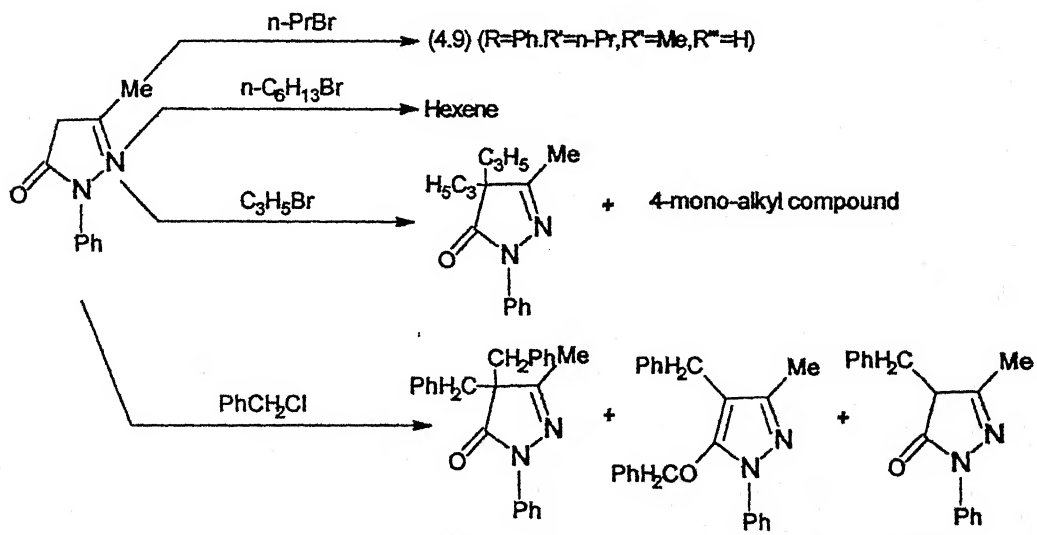


(4.8)

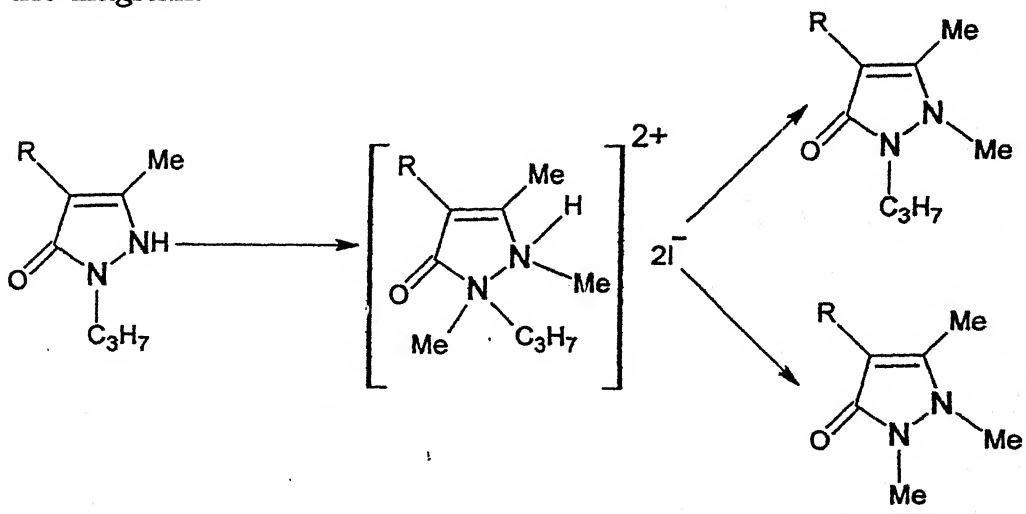


(4.9)

The ethylation of 3-methyl-1-phenylpyrazol-5-one was early described **[71]**, but attempts to prepare higher homologues of antipyrine gave a variety of results **[146]**, shown below. *C*-Alkylations occurring under other conditions are also shown here.

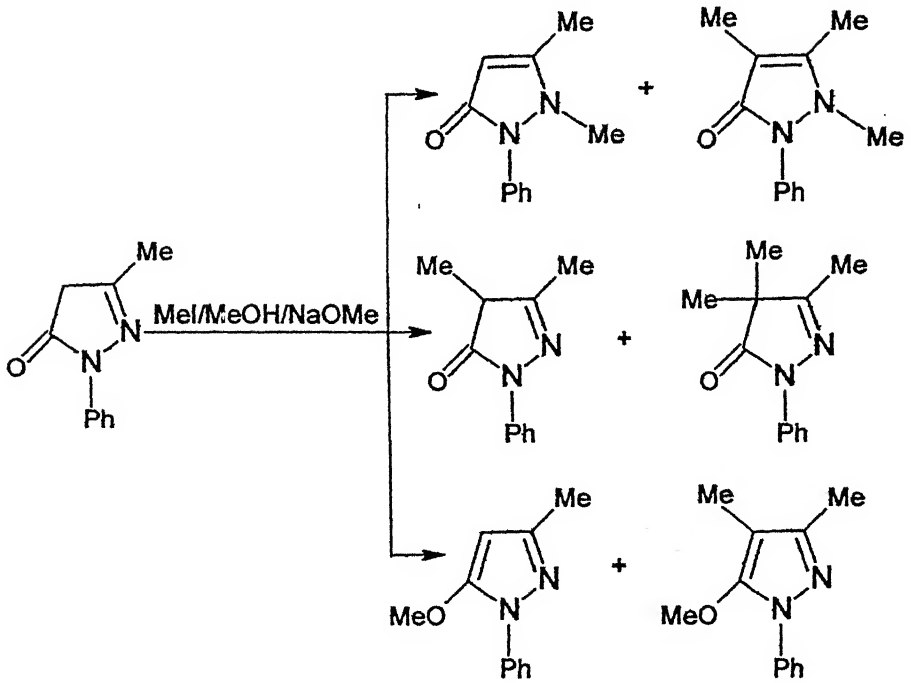


Isoantipyrine (4.8), R = R/= Me, R//= Ph, R///= H, is formed by heating 1-methyl-3-phenylpyrazol-5-one with methyl iodide **[143d]**. In general the 2-methylation of 1-alkylpyrazol-5-ones **[139g,78a]** is more difficult than that of 1-arylpyrazol-5-ones, and exchange of alkyl groups can occur **[78a]**. Thus, when 3-methyl-1,4-di-isopropylpyazol-5-one is heated with methyl iodide it seems that some 1,2,3-trimethyl-4-isopropylpyrazol-5-one is formed, and 3-methyl-4-phenyl-1-isopropylpyrazol-5-one gives the 1,2,3-trimethyl compound, though 1-ethyl-3-methyl-4-isopropylpyrazol-5-one gives the 1-ethyl-2,3-dimethyl compound. The exchanges have been represented as shown in the diagram.



Methylation of 3-methyl-1-phenylpyrazol-5-one, other than by heating with an alkyl halide, was first described by Knorr; the use of methyl iodide with sodium methoxide was said to effect the successive introduction of two methyl groups at C-4. 3,4-Dimethyl-1-phenylpyrazol-5-one similarly gave 3,4,4-trimethyl-1-phenylpyrazol-5-one **[71k]**. Later, the six products shown were

reported to arise from the methylation of 3-methyl-1-phenylpyrazol-5-one under these conditions **[71f]**.



Other workers **[147]**, using sodium methoxide, methanol, and dimethyl sulphate, prepared 5-methoxy-3-methyl-1-phenylpyrazole, and from the pyrazolone in boiling sodium hydroxide solution by adding dimethyl sulphate, a high yield of antipyrine. *C*-and *O*-benzylations, using sodium ethoxide and benzyl chloride, have been described **[146]**, and *p*-nitrobenzyl chloride effects 4-substitution **[129b]**. Ethyl chloroacetate and sodium ethoxide give *O*-substitution **[149e]**.

3-Methyl-1-phenylpyrazol-5-one is substituted at C-4 by reaction with chloroacetone and sodium hydroxide, and this **[148c]** and other **[148b]** 4-substituted compounds give analogues of antipyrine when methylated in presence of alkali. Similarly 3-

methyl-1-phenyl-4-isopropylpyrazol-5-one with alkyl halides, sodamide, and dioxan, is said to give analogues of antipyrine **[78]**.

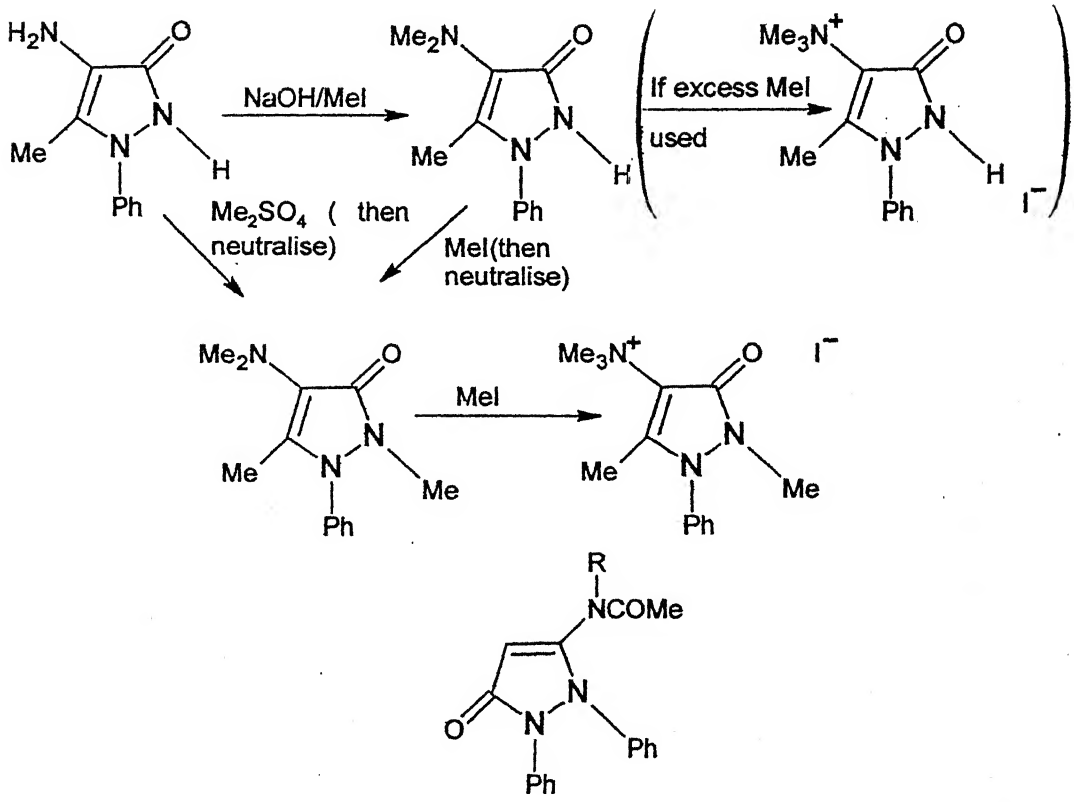
1-Phenylpyrazol-3-one is *O*-methylated with dimethyl sulphate and aqueous alkali **[150]** and gives the *O*-ether with allyl bromide and potassium carbonate in acetone **[150]**, but with aqueous alkali a 35% yield of 2-allyl-1-phenylpyrazol-3-one **[151]**.

In contrast to these base-catalysed alkylations is the 4-alkylation of 3-methyl-1-phenylpyrazol-5-one with triarylcarbinols or their ethers in acetic acid containing hydrochloric acid **[152a,b]**.

Diazomethane has not been much used with pyrazolones, but some 3-methyl-1-thiazolylpyrazol-5-ones are reported to give analogues of antipyrine with this reagent **[153]**. In contrast, 3-methyl-1-phenylpyrazol-5-one gives mainly the *O*-methyl ether, with only a trace of antipyrine **[154b]**.

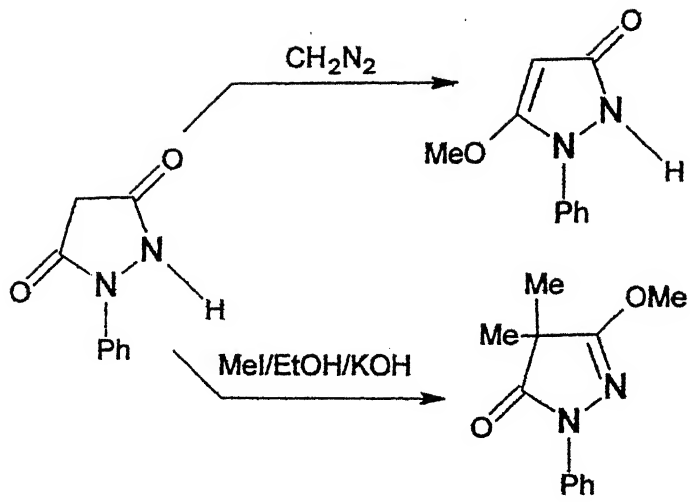
The behaviour of 4-amino-5-methyl-1-phenylpyrazol-3-one on methylation is shown below **[143c]**. With alkyl halides, 4-aminoantipyrine gives the 4-alkylamino compounds **[139a,b,e,g,71m,138b,142b,145d]**. Such reactions were and remain important because of the value of the products as drugs; 4-dimethylaminoantipyrine ('Aminopyrine', 'Pyramidone') was first prepared by Stolz **[139a,b,712m,138b]**, by reaction of aminoantipyrine with methyl iodide in methanolic potash.

The acylaminopyrazolone (4.10), R = H, gives (4.10), R = Me, with diazomethane **[155]**.



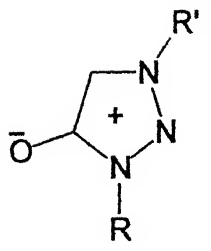
(4.10)

In the alkylation of 1-phenylpyrazol-3,5-diones both carbon and oxygen compete successfully against nitrogen **[76a,156b]**, as shown, and alkylation at C-4 is generally observed **[157f]**. 4-Butyl-1,2-diphenylpyrazol-3,5-dione('Phenylbutazone') was introduced in 1946 for use against rheumatoid arthritis; it and its homologues are readily prepared by reaction of 1,2-diphenylpyrazol-3,5-dione with alkyl halides and alkali **[158,159a-c]**. 4,4-Dialkylation can also be effected **[160]**.

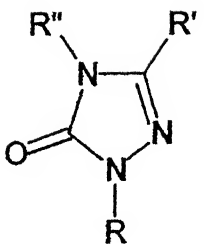


Probably because the products lack the practical value of the pyrazolones, the alkylation of imidazolones has been very little investigated. With alkali and dimethyl sulphate, imidazol-2-one-4-carboxylic acid is methylated at both nitrogen atoms **[161]**. With methanolic potash and methyl iodide, hydantoin gives 3-methylhydantoin **[162e,163]**, and diazomethane methylates 1-ethylhydantoin at N-3 **[164]**.

Recently the methylation of hydroxy-1,2,3-triazoles with diazomethane has been reported. Other reagents have been much less used. The results (Table 4.3) show that attempts to monomethylate a compound initially un-substituted on nitrogen fail, dimethyl compounds always being formed. Commonly, whether the starting compound was substituted on nitrogen or not, all of the possible *O,N*-dimethyl compounds were formed. The invariable formation in appropriate cases of the meso-ionic derivative (4.11) as an important proportion of the total products is interesting.



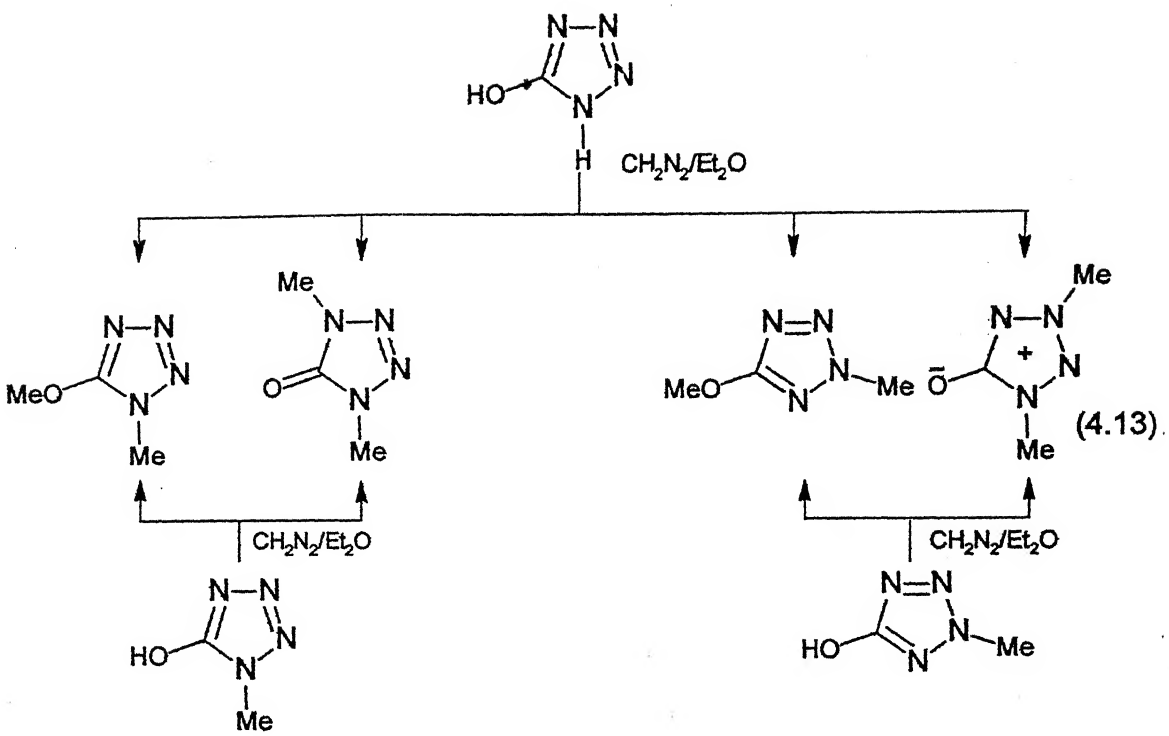
(4.11)



(4.12)

According to the conditions, methylation in the presence of base of 5-nitro-1,2,4-triazol-3-one can give either the 1- or the 4-methyl compound **[165]**. Alkylation in the presence of bases of some 1-substituted 5-hydroxy-1*H*-1,2,4-triazoles **[166a,167e]** and some 4-substituted 5-hydroxy-4*H*-1,2,4-triazoles **[168e, 162d,169b]** has been represented as giving products of the type (4.12). This orientation has been proved for the compound (4.12), R = Me, $R^{/}$= H, $R^{//}$= NH_2, derived from (4.12), R = $R^{/}$ = H, $R^{//}$= NH_2, and is probably correct in analogous cases **[168e]**, but with other compounds it is not clear that isomeric structures can be excluded.

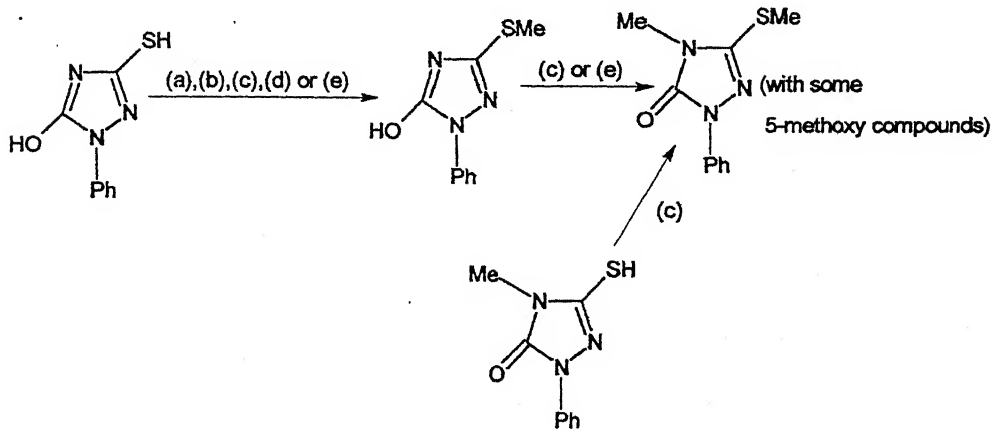
The reactions of 5-hydroxytetrazole and its derivatives with diazomethane are illustrated **[125,229]**. All of the possible dimethyl compounds except one appear to be formed; the structure of the product represented as (4.13) (a trace of which is also formed from 5-hydroxy-1-methyl-tetrazole) is probable but not completely established. With ethyl bromoacetate in the presence of triethylamine, 5-hydroxytetrazole gave only the 1,4-disubstituted tetrazolone **[134]**.



(4.13)

A number of observations have been made concerning the alkylation of azol-thiones. Various 1-aryl-3-methylpyrazol-5-thiones react at the sulphur atom with methyl iodide **[143f]**. Similarly a number of *N*-unsubstituted imidazol-2-thiones have been alkylated on the sulphur atom under conditions which include heating with an alkyl halide **[173b]**, or with alcoholic hydrogen chloride **[174c]**, and heating the alkylating agent with the sodium salt of the thione in water **[175]** or liquid ammonia **[176b]**. 4-Phenylimidazol-2-thione with dimethyl sulphate and alkali gave the *S*-methyl compound and both possible *N,S*-dimethyl compounds **[177]**. 1-Methyl- and 1-phenyl-imidazol-2-thione give the methylthio compounds when treated with methyl iodide in chloroform, or with dimethyl sulphate and aqueous potassium carbonate **[178,179]**.

Similarly, *N*-unsubstituted 1,2,4-triazol-5-thiones give methylthio compounds when heated with methyl iodide **[180a]**, whilst with *N*-unsubstituted compounds methyl iodide **[180b]** or an alkylating agent and a base **[179,181a-c]** have been used. 5-Hydroxy-1-phenyl-1,2,4-triazol-3-thione(1-phenyl-3-thiourazole) has been alkylated as shown above **[182a]**.



(a) MeOH/HCl

(b) MeI+Ag salt

(c) MeI+Na salt

(d) MeI+ K salt

(e) CH_2N_2

Alkylation of the sulphur atom rather than a nitrogen atom in 1-substituted tetrazol-5-thiones is also generally observed. Reagents used have been alkyl halides in aqueous alcohol **[183c]**, alkyl halides with sodium ethoxide **[184a,124d]** or sodium hydroxide **[183b]**, dimethyl sulphate and potassium carbonate **[179]**, the silver salt with methyl iodide **[184a]**, and diazomethane **[124d]**. In the Mannich reaction 1-aryltetrazol-5-thiones give what are probably the 4-dialkylaminomethyl compounds **[183a]**.

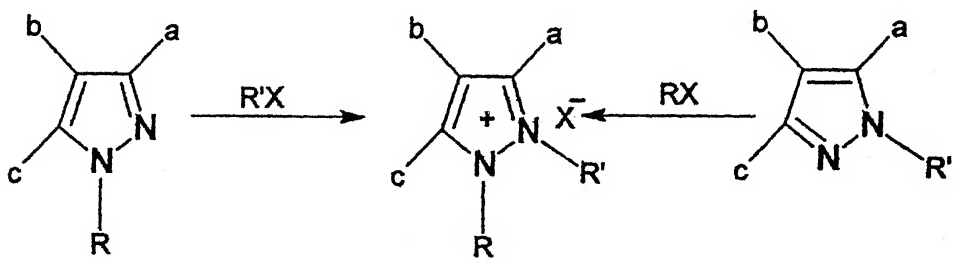
(ii) The Attachment of Alkyl and Substituted Alkyl Groups: Quaternising Alkylation :

The incidental formation of quaternary salts during the substitutive alkylation of pyrazoles has been mentioned; it happened in the reaction of pyrazole with methyl iodide **[69f,70a]**. Knorr and Kohler **[71j]** who prepared 1-methylpyrazole methiodide by heating pyrazole with methyl iodide in methanol, proved it to be 1,2-dimethylpyrazolium iodide by degrading it to 1,2-dimethylhydrazine. Commonly quaternary pyrazolium salts have been prepared by heating at about 100°C a mixture of a *N*-substituted pyrazole and an alkyl halide. The earliest examples seem to have been the quaternisation with methyl iodide of 1-phenyl- and 4-methyl-1-phenyl-pyrazole **[185d]**. 5-Chloro-3-methyl-1-(*m*-nitrophenyl)-pyrazole was quaternised with dimethyl sulphate **[143h]**. The structures of the salts from 1-phenylpyrazole and methyl and ethyl iodide as well as of 1,2-diethyl- and 1-ethyl-2-methylpyrazolium iodides, were proved by degradation to hydrazines **[186b]**. 3,4,5-Trimethyl-1-phenylpyrazole **[187d]** and 1-alkyl- or -aralkyl-pyrazoles containing alkyl groups **[64a,d,e,65f,66c]**, chloro and methyl groups **[66a,d]**, and methoxycarbonyl and methyl groups **[65f]** have been quaternised without difficulty. The examples illustrated, like the degradations already mentioned, show that a 1-substituted pyrazole is quaternised at N-2. Other examples are found among the 3,4-tetramethylene-pyrazoles (tetrahydro-indazoles) **[64a,d,e]**.

Reactions in which *N*-alkylpyrazoles are converted into different *N*-alkylpyrazoles by being heated with an alkyl halide go through quaternary salts.

As in the pyrazole series so with the imidazoles, quaternisation to some degree has often accompanied substitutive alkylation. The corresponding quaternary salts were obtained by boiling imidazole with ethyl bromide or benzyl chloride **[88b]**, or with methyl iodide, chloracetic acid or ethyl chloracetate **[93]**.

Numerous examples have been described of the quaternisation of 1-alkylimidazoles with alkyl, alkenyl, or aralkyl halides **[90a,91c,e,.d,65h,92c,188a,93,189,190b]** ethyl chloracetate **[93]**, or phenacyl bromide **[191]**.



R = R′ = a = Me; *c* = Cl; *b* = H **[66a]**

R = CH_2Ph; R′ = *a* = Me; *b* = H; *c* = Cl **[66a]**

R = *a* = Me; *b* = H; *c* = Cl; R′ = CH_2Ph **[66a]**

R = *c* = Me; a = b = H; R′ = CH_2Ph **[65f]**

R = CH_2Ph; *a* = *b* = H; *c* = R′ = Me **[65f]**

R = *c* = R′ = Me; *a* = CO_2Me; b = H **[65f]**

R = *c* = R′ = Me; *c* = *b* = H **[65f]**

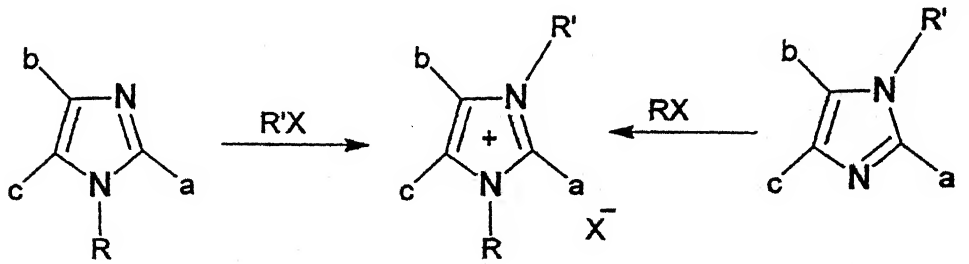
R = *a* = *b* = R′ = Me; *c* = Cl **[66d]**

R = *a* = R′ = Me; *b* = Cl; *c* = H **[66b]**

R = *a* = R′ = Me; *b* = NO_2; *c* = H **[66d]**

N-Alkylimidazoles containing aryl **[98]**, halogen **[188a,c,174b]**, and nitro groups **[98]** have also been quaternised. The reactions occur readily; 1-methyl- or 1-ethyl-imidazole and methyl iodide react together most vigorously **[192,91d]**. *N*-Arylimidazoles have been quaternised by fusing with methyl toluene-*p*-sulphonate **[193b]**.

The first evidence to demonstrate that quaternisation of a 1-alkyl-imidazole proceeds at the unsubstituted nitrogen atom was obtained by Pinner and Schwarz **[92c]**, who showed that the quaternary salt from 1-methylimidazole and amyl bromide was decomposed by alkali to give both methylamine and amylamine. Other such examples were described. Later authors observed the formation of the same quaternary salt from different alkylimidazoles : see diagram.



R = *b* = R/ = Me; *a* = c = H **[90]**

R = Me; R/ = n-Pr; *a* = *b* = c = H **[65h]**

R = R/ = Me; *b* = Cl; *a* = *c* = H **[188a]**

R = R/ = Me; *b* = NO_2; *a* = *c* = H **[98]**

R = R/ = Me; *b* = Br; *a* = *c* = H **[174b]**

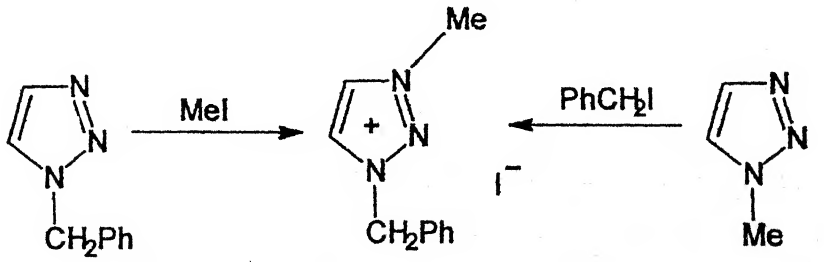
R = R/ = Me; *b* = Ph; *a* = *c* = H **[98]**

R = Et; R/ = n-Pr; *a* = *b* = *c* = H **[65h]**

The rates of reaction of some 1-substituted imidazoles with ethyl iodide in ethanol **[194]** and in acetone have been measured **[195]**. The expected sequence of reactivities, 1-Me > 1-$PhCH_2$ > 1-Ph is observed. Only qualitative observations have been recorded regarding substituent effects; thus, 1-methyl-4- and -5-chloroimidazole react readily enough with methyl iodide but less easily than does 1-methylimidazole **[188a]**.

Quaternisations of imidazoles in which the initial *N*-substituent is other than an alkyl, aralkyl, or aryl group are known. Thus 1-benzoyl-4-phenylimidazole is quaternised with triethyloxonium tetrafluoroborate. Methanolysis of the product gives 1-ethyl-5-phenylimidazole **[196]**. The derivative of 2-methylimidazole with the group –PS(Ph}.NEt_2 attached to nitrogen is quaternised on the ring with methyl iodide **[197]**.

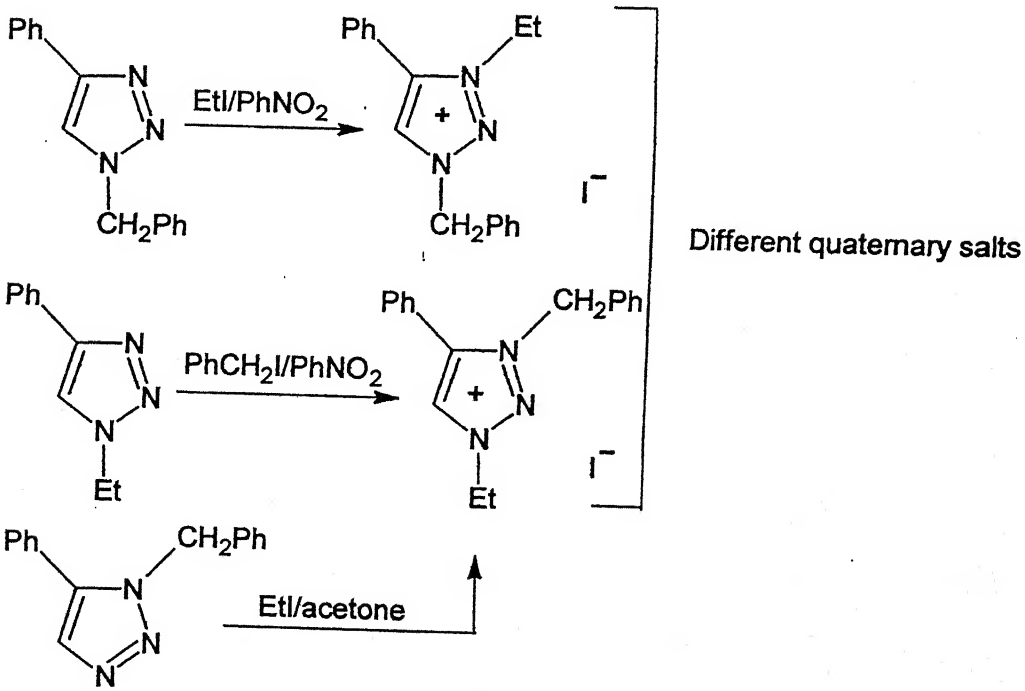
Wolff **[136c]** prepared the first quaternary salt of the 1,2,3-triazole series by heating 1,5-dimethyl-1*H*-1,2,3-triazole with methyl iodide at 100°C. Much later **[198b]** the first reactions we show were regarded as demonstrating that quaternisation of 1,2,3-triazoles proceeded at N-3.



The quaternary salt was said to be homogeneous, but if the experiments excluded a 1,2-substituted structure for the cation,

they did not strictly exclude the 1,1-disubstituted structure. This unlikely possibility was removed by the reactions shown next **[86a]**.

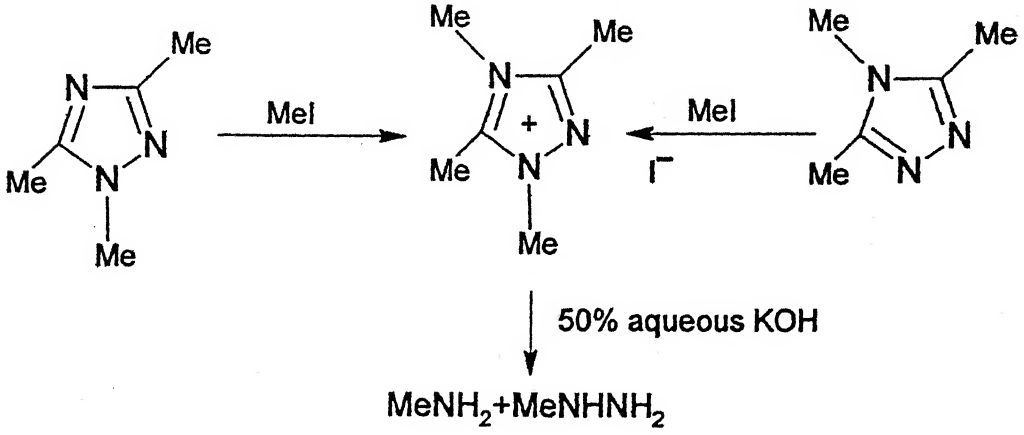
1-Phenyl-1*H*-1,2,3-triazole reacted with methyl iodide in acetone-ether during 2-5 weeks at room temperature **[199]**. It is never clear from these various reports whether the frequently longer times or more severe conditions of reaction used (compared with those used for diazoles) are necessary for successful quaternisation of 1,2,3-triazoles.



1-Substituted 1*H*-1,2,3-triazoles can be quaternised with methyl tolune-*p*-sulphonate, but the 2-substituted compounds do not react with the reagent, with methyl iodide or dimethyl sulphate, or merely give very low yields of products. In contrast,

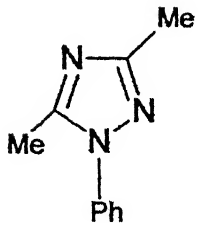
2-methyl- and 2-phenyl-2*H*-1,2,3-triazole are both efficiently quaternised with methyl fluorosulphonate (n.m.r. spectroscopy shows the two groups attached to nitrogen to be on different nitrogen atoms) **[120k]**.

Early workers quaternised several 1-aryl-1*H*-1,2,4-triazoles, containing *C*-alkyl **[200a, 201-02]** or *C*-aryl groups **[203]**, with methyl or ethyl iodide, without being able to assign structures to the products. Correct proof of the structure of a quaternary salt in this series was obtained as illustrated.

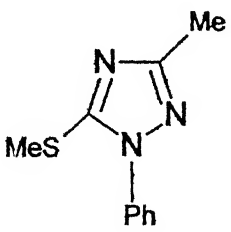


The salt was degraded to methylamine and methylhydrazine, and similar evidence proved the product from 3,5-dimethyl-1-phenyl-1*H*-1,2,4-triazole and methyl iodide to be 3,4,5-trimethyl-1-phenyl-1,2,4-triazolium iodide **[181a]**. The same general mode of quaternisation is in other cases attested by evidence of a different kind. Thus, the salts formed from methyl toluene-*p*-sulphonate and both (4.14) and (4.15) with the appropriate second components give the same cyanines, proving the salt from (4.14) to be (4.16) **[181a]**; a *C*-methyl group in such compounds is only

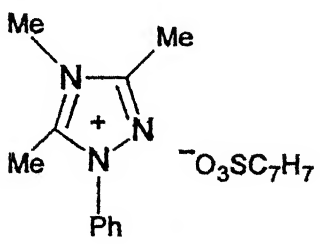
reactive when situated between the two substituted nitrogen atoms . **[138g, 181a]**. The quaternary salts (4.17) from 3-methyl-1-phenyl-1*H*-1,2,4-triazole do not show reactivity of the *C*-methyl group. This circumstance is further illustrated by the properties of the compound obtained by treating the sodium salt of 3-methyl-1,2,4-triazole with triphenylmethyl chloride (Table 4.2); with methyl iodide this give a quaternary salt which does not react with *p*-nitrosodimethylaniline **[204a]** and is, therefore, probably (4.18).



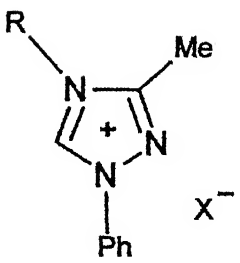
(4.14)



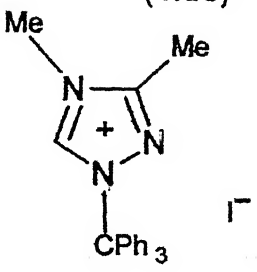
(4.15)



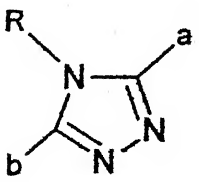
(4.16)



(4.17)



(4.18)



(4.19)

It can then generally be assumed that 1-substituted 1*H*-1,2,4-triazoles are quaternised at N-4 (thus, the product from 1-dodecyl-1*H*-1,2,4-triazole and ethyl iodide is probably 1-dodecyl-4-ethyl-1,2,4-triazolium iodide **[205]**), and that 4-substituted 4*H*-1,2,4-triazoles are quaternised at N-1 or N-2 (thus, 4-aryl-3,5-

dimethyl-4*H*-1,2,4-triazoles give 4-aryl-1,3,5-trimethyl-1,2,3-triazolium iodides **[181a,190c]**, but obviously triazoles of the type (4.19) could still give two quaternary salts. If either *a* or *b* is a methyl group its properties in the quaternary salt give evidence for the structure of the latter, as illustrated by the examples containing sulphur substituents which are quoted below.

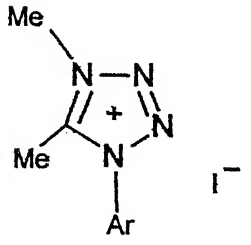
The quaternary 1,2,4-triazolium salts so far mentioned were prepared using alkyl halides or toluene-*p*-sulphonates as quaternising agents. Triethyloxonium tetrafluoroborate in ethylene chloride has been used to quaternise 5-chloro-3-methyl-1-phenyl-1*H*-1,2,4-triazole **[206]**. This last reagent, in nitromethane, converts 1,2,4-triazole itself into a mixture of both possible *N*-methyl compounds and the 1,4-dimethyl quaternary salt **[196]**. It also converts 1-acetyl-1*H*-1,2,4-triazole into 1-acetyl-4-methyl-1,2,4-triazolium tetrafluoroborate which, on methanolysis, give 4-methyl-4*H*-1,2,4-triazole **[196]**.

A number of 1,2,4-triazolium salts have been prepared from 3-arylazo-1,2,4-triazoles or the corresponding 5-carboxylic acids by combined substitutive and quaternising methylation, e.g. by reaction with dimethyl sulphate in *o*-dichlorobenzene or dimethylformamide, sometimes in the presence of magnesium oxide **[138i,159d,169a,c,190,e ,h,i,j,207]**.

An early attempt to quaternise 2,5-diphenyl-2*H*-1,2,3,4-tetrazole by heating it at 100°C for 3 hours with methyl iodide failed **[208a,237-38]**. In similar conditions 5-methyl-1-(3,4-dimethylphenyl) tetrazole and related compounds were

quaternised with methyl iodide, neat or in boiling isopropanol or with methyl benzenesulphonate on the steam-bath **[209-10]**.

The structures of the quaternary salts from 5-methyl-1-phenyl- and 5-methyl-1-(3,4-dimethylphenyl) tetrazole with methyl iodide were proved to be (4.20.a) and (4.20.b) respectively by alkaline degradation to methylamine and an aryl



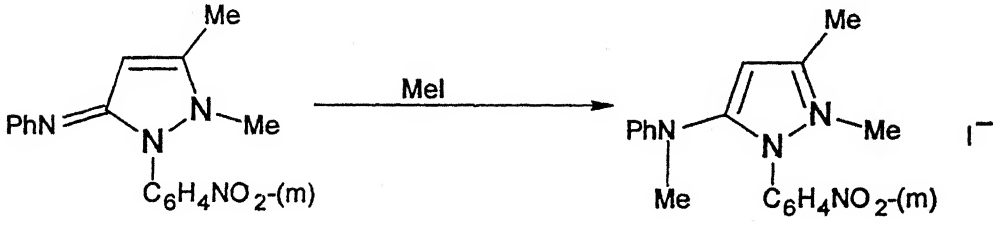
(4.20) a,Ar = Ph; b, $C_6H_3Me_2$ (3,4)

azide **[181b]**. Several other related quaternary salts, as well as 1,4,5-trimethyltetrazolium iodide, have been described **[190d]**. Ring-opening reactions prove the salt from 1-ethyltetrazole and ethyl toulene-*p*-sulphonate to be 1,4-diethyltetrazolium toulene-*p*-sulphonate **[211b,231-32]**, and that from 2-methyltetrazole and methyl benzenesulphonate to be 1,3-dimethyltetrazolium benzenesulphonate **[212a,234-35]**. The general presumption from these results is that 1-and 2-substituted tetrazoles are both quaternised at N-4. However, this is not completely true; 1-methyl-5-phenyl-1*H*-1,2,3,4-tetrazole kept with methyl iodide for 90 days at room temperature gives starting material (55%), 2-methyl-5-phenyl-2*H*-1,2,3,4-tetrazole (6%), and a mixture of 1,3-dimethyl- and 1,4,dimethyl-5-phenyl-1,2,3,4-tetrazolium salts (37%). The relative stabilities of the two quaternary salts lead to

the production from the reaction components of 2-methyl-5-phenyl-2*H*-1,2,3,4-tetrazole at higher temperature.

We shall now consider the quaternisation of azoles containing substituents which can compete with the ring nitrogen atom for the alkylating agent.

5-Amino-1-arylpyrazoles are quaternised at N-2 by alkyl halides **[143b, 213c,d]**. As in the pyridine series the anhydronium or conjugate bases of the quaternary cations are quaternised at the exocyclic nitrogen atom, as in the example shown **[143h]**.



In contrast to the 5-amino compounds, 4-amino-5-chloro-3-methyl-1-phenylpyrazole reacts with methyl iodide to give 5-chloro-4-dimethyl-amino-3-methyl-1-phenylpyrazole hydriodide, and the free base from this salt is quaternised by methyl iodide at the exocyclic nitorgen atom **[76f, 213e]**.

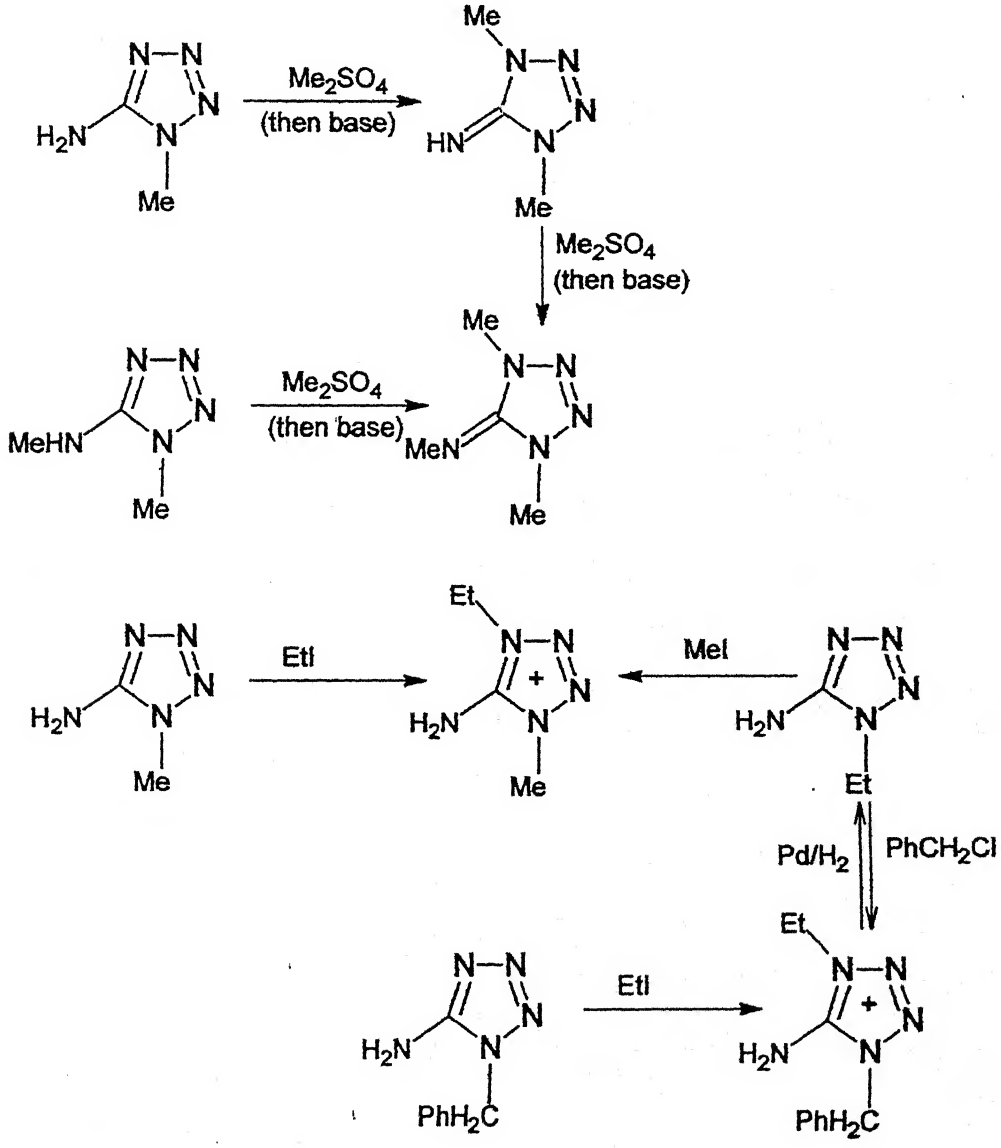
Little is known about the quaternisation of amino-imidazoles or aminotriazoles. 5-Amino-4-aminocarbonyl-1-benzyl-1*H*-1,2,3-triazole is quaternised at N-3 by methyl toluene-*p*-sulphonate **[121b]**, and 4-amino-4*H*-1,2,4-triazoles are quaternised at N-1 **[214a]**. 5-Amino-1-methyl-1*H*-1,2,4-triazole is quaternised at N-4 **[215d]** .

As indicated above alkylating agents reacting with 5-aminotetrazole in the absence of alkali effect both substitutive and quaternising alkylation. The reaction of the amine with alkyl halides was early studied **[129c]**, though correct identification of the products took a long time **[130e]**. At one stage the products formed when various 1-alkyl-5-amino- and 5-amino-1-aryl-1*H*-1,2,3,4-tetrazoles were heated with alkylating agents (methyl benzenesulphonate, dimethyl sulphate, diethyl sulphate, and alkyl halides) were believed to be 1-substituted 5-alkylaminotetrazoles **[130a]**. The differences in properties of these products and compounds unambiguously possessing such structures **[216a]** showed this view to be wrong, and a 1-alkyl–5–aminotetrazole heated with and alkylating reagent gives, in fact, a 1,4-dialkyl-5- aminotetrazolium salt. Usually the reaction mixture is basified and the product isolated as the anhydronium base, a 1,4-dialkyl-5-iminotetrazole **[130c]**. These formulations are supported by sequences such as those illustrated **[217,130c]**. In fact, ring alkylation occurs, generally at both possible sites, as in the next cases shown **[127]**. The structure of (4.21) is adopted from the proved **[126]** structure of (4.22). The formation of 1,2-dialkyl-5-iminotetrazoles has not been observed.

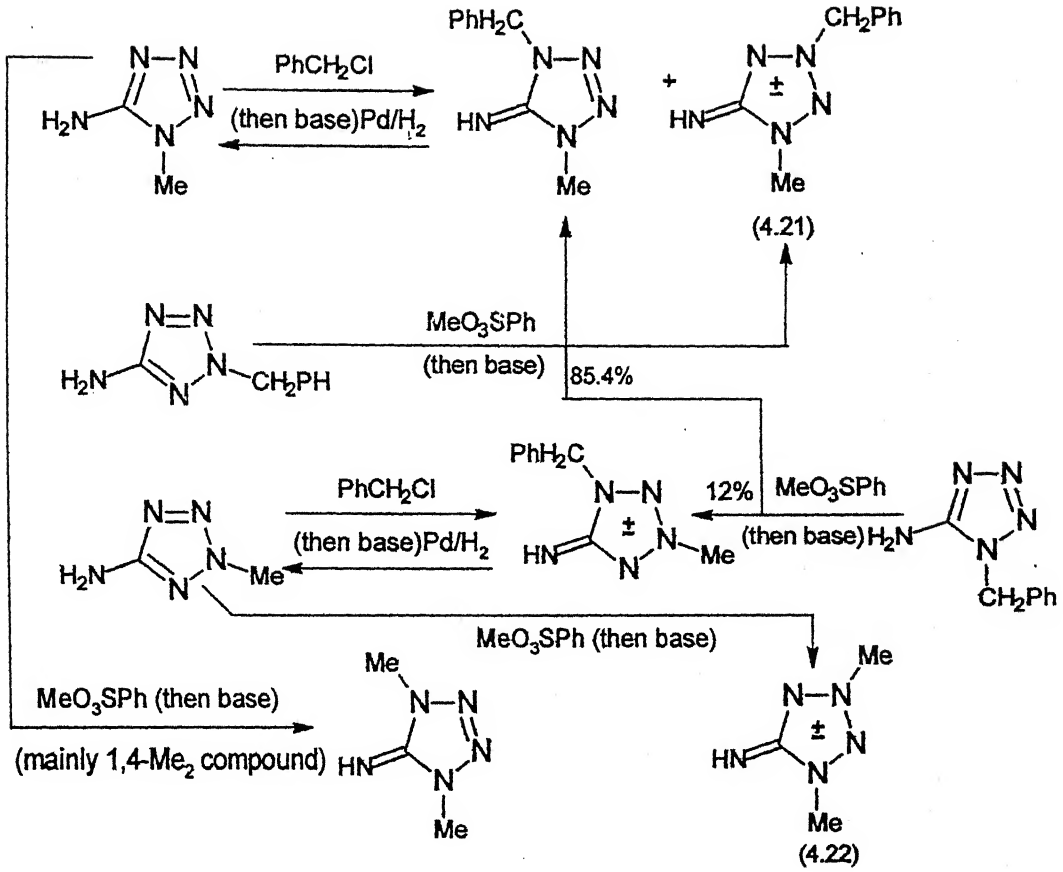
As illustrated in one instance above, further quaternisation of 1,4-dialkyl-5-iminotetrazoles proceeds, as would be expected, at the exocyclic nitrogen atom. In another example 1-benzyl-4-methyl-5-iminotetrazole gave with methyl benzenesulphonate the

quaternary salt from which basification produced 1-benzyl-4-methyl-5-methyliminotetrazole **[127]**.

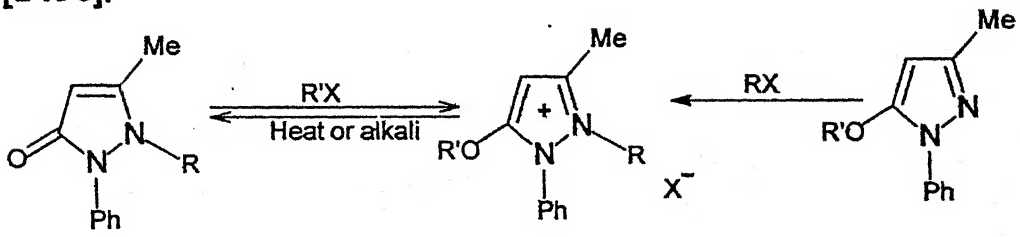
Numerous1-alkyl-5-aminotetrazoles have been quaternised by heating with alkylating agents, and the products identified by their preparation from two different 1-alkyl compounds reacting with complementary alkylating agents, or by hydrogenolysis of benzyl groups as illustrated above **[130e,f,g]**.



When we turn to derivatives of hydroxyazoles we find some complicated results in the pyrazolone series, involving the quaternisation of antipyrines. In the simplest circumstances a quaternary salt arises from the reaction of antipyrine (or an analogue) with an alkyl halide at 60°C, or more slowly at room temperature. The structures illustrate the situation. Experiments at higher temperatures produce more complicated results; antipyrine with methyl iodide at 80-200°C, or (4.23) R = R/= Me, treated similarly, gives 4-methylantipyrine and 3,4,4-trimethyl-1-phenylpyrazol-5-one. The proportion of the trimethyl compound increases with temperature; it does not arise by the direct isomerisation of 4-methylantipyrine, the presence of methyl iodide being necessary. Next shown are reactions related to these **[186c]**. In the case where (R = H) later workers detected also the formation of some 4-benzyl-3-methyl-1-phenylpyrazol-5-one **[146]**. The *C*-alkylations have been attributed to the formation of quaternary salts by attack of the alkylating agent at N-2 with subsequent migration to C-4; loss of the *N*-benzyl group from such a quaternary salt generates an antipyrine with methyl in place of benzyl **[146,186c]**.



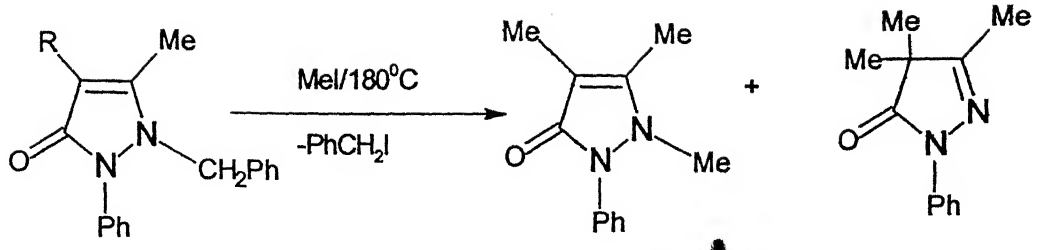
Acyloxy- and alkoxy-pyrazoles are quaternised at nitrogen **[149e]**.

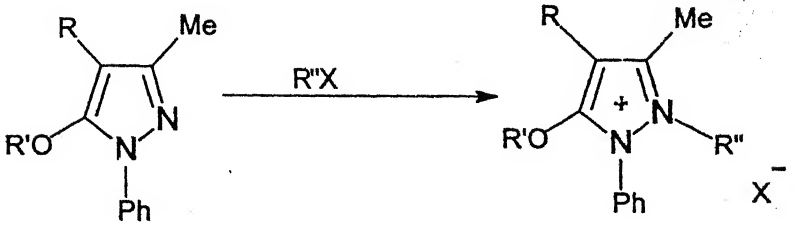


(4.23)

R = R/ = Me

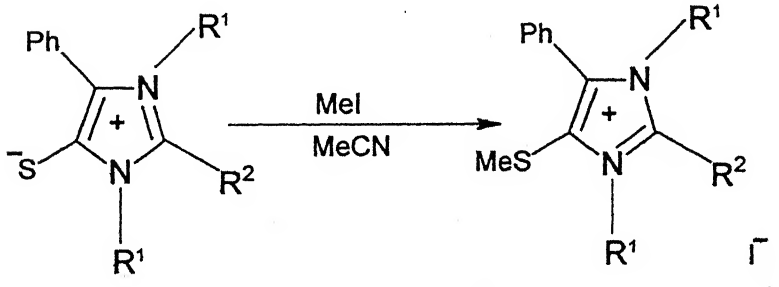
or R = Me, R/ = Et





R = H, R/ = PhCO,R//X = MeI

R = Me, R/= Et, R//X = $BrCH_2CO_2Et$

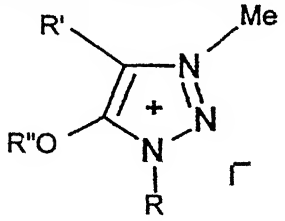


$R^1 = R^2 = Me$; $R^1 = C_4H_9$,$R^2 = CHMe_2$

As illustrated here, meso-ionic sulphur derivatives of imidazole form quaternary salts with methyl iodide **[218]**.

In the 1,2,3-triazole series, quaternary salts (4.24), R = Me, R/= H, R// = Me or Et, have been obtained from meso-ionic compounds of the type (4.11) by reaction with methyl or ethyi iodide **[118b]**, but attempts to obtain the quaternary salts from several other meso-ionic compounds of this type failed **[120c]**. This is not surprising in view of the reactions which occur between 1-substituted 5-methoxy-1,2,3-triazoles and alkyl

halides (Table 4.4). Commonly, meso-ionic compounds of the type (4.11) are formed, no intermediate quaternary salt being detectable or isolable. In a few cases, however, the quaternary intermediates have been either detected or isolated (Table 4.4).

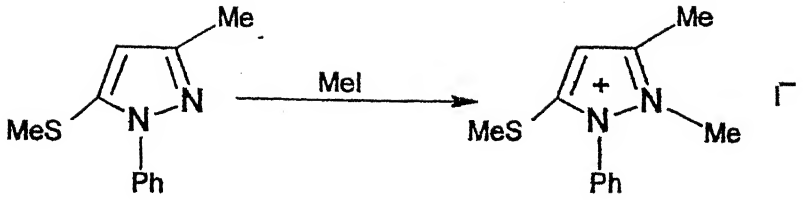


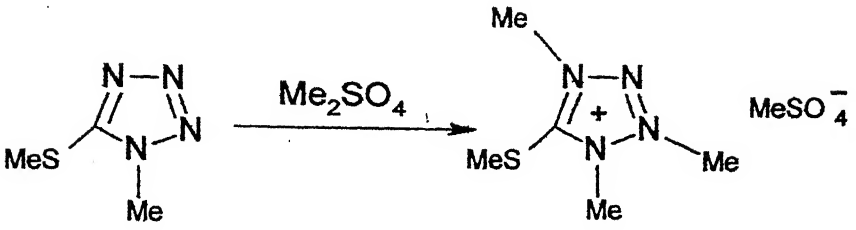
(4.24)

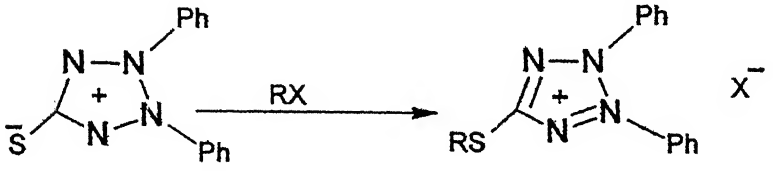
Numerous examples of the formation of quaternary salts from the sulphur analogues of antipyrine have been described. As would be expected, these reactions, some of which are given in Table 4.5, occur at the sulphur atom, whilst the methylthio compounds react at nitrogen, as shown here **[143f]**. The related selenium compounds behave similarly **[143a,d]**.

Sulphur derivatives of 1,2,4-triazole show the same pattern of behaviour, but in the cases where reaction occures at nitrogen the problem of orientation arises. This can be solved by noting the effect of the quaternisation upon the reactivity of *C*-attached substituents (Table 4.6).

Two related reaction in the tetrazole series are shown **[179,219]**.







RX = MeI and $ClCH_2COOH$

(iii) The Attachment of Aryl Groups : Substitutive Arylation:

Azoles have been successfully *N*-arylated by reaction with halogeno-benzenes, activated towards nucleophilic attack, under a variety of conditions, most commonly in the absence of strong bases .

There are several examples of the *N*-arylation of imidazole by unactivated halogenobenzenes in the presence of cuprous bromide; these reactions may differ mechanistically from the others.

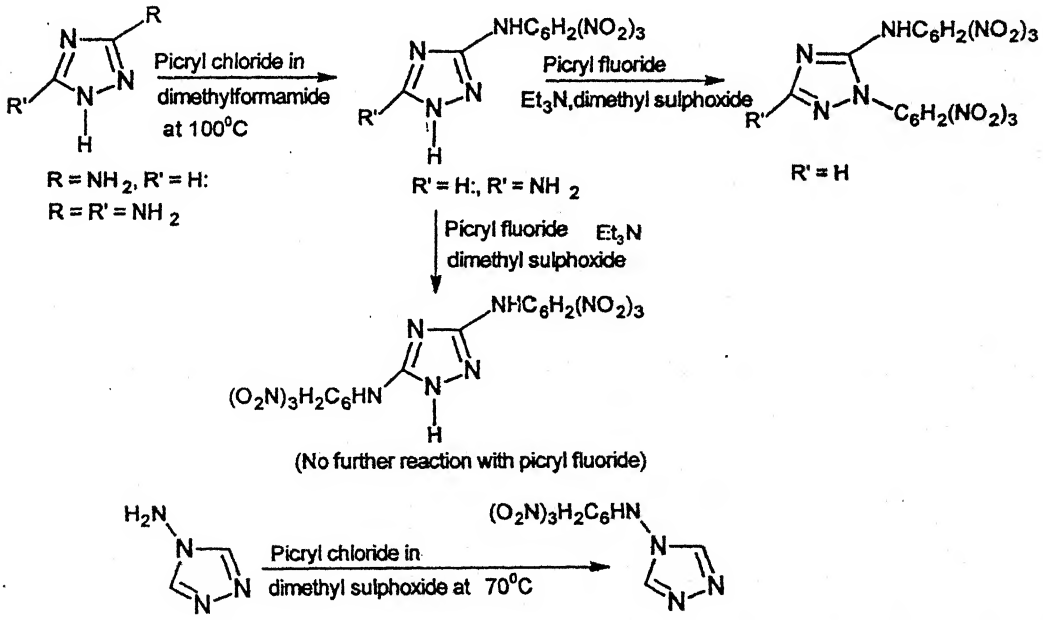
In the pyrazole series the less basic the compound the greater was the reaction time, and with substituted pyrazoles the least hindered product appeared to be formed preferentially **[220b]**. 3,5-Di-t-butyl-,3,5-di-t-butyl-4-methyl- and 4-t-butyl-3,5-

dimethyl-pyrazole did not react with 2,4-dinitrofluorobenzene either in boiling ethanol or boiling xylene **[220b]**.

N-Picrylpyrazoles have been prepared in good yield by heating together picryl chloride and *N*-acylpyrazoles **[221]**. 1-Acetyl-3-methyl-pyrazole gives 3-methyl-1-picrylpyrazole.

Not much work has been reported on the arylation of azoles containing tautomerisable substituents. 3-Amino-1,2,4-triazoles react first at the amino group with picryl chloride, as does 4-amino-4*H*-1,2,4-triazole **[222a]** see diagram.

With 2-bromopyridine and related heterocycles, the sodium salt of 2,3-dimethylpyrazol-5-one gives 3-aryloxy-1-5-dimethylpyrazoles rather than *N*-arylated products **[223]**.



4.2 : Experimental :

(a) Materials Employed:

1,2,4-Triazole, 3-Amino-1,2,4-triazole, 5-Methyl benzotriazole and 5-Nitro benzotriazole were procured from Aldrich Chemical Company, U.S.A. and used as such $PtCl_2$ and Chemical 1,2,4-Triazole, 3-Amino-1,2,4-triazole, 5-Methyl benzotriazole and 5-Nitro benzotriazole were obtained from TOKYO KASEI Organic Chemical, Japan and B.D.H England. Distilled water used in all the operation.

(b) Preparation of the Coordination Compound :

(i) Preparation of the Coordination Compound [Pt(1,2,4-TAZ)$_2$Cl$_2$] :

A mixture of $PtCl_2$ (500mg) and ligand 1,2,4-triazole (1gm) in water and methanol (50ml) was refluxed at 80^{0}C 5-6 hours until it became a clear gray colour solution. This volume was reduced to 5ml and treated with methanol. The resulting gray crystals were collected and washed well with ethanol and acetone. The analytical data are given in Table 4.7.

(ii) Preparation of the Coordination Compound [Pt(3-Amino-1,2,4-TAZ)$_2$Cl$_2$] :

A mixture of $PtCl_2$ (500mg) and ligand 3-amino-1,2,4-Triazole (1gm) in water and methanol (50ml) was refluxed at 80^{0}C 5-6 hours until it became a clear gray-white colour solution. This volume was reduced to 5ml and treated with methanol. The resulting gray-white crystals were collected and washed well with ethanol and acetone. The analytical data are given in Table 4.7.

(iii) Preparation of the Coordination Compound [Pt(5-methyl-BZT_R)$_2Cl_2$] :

A mixture of $PtCl_2$ (500mg) and ligand 5-methyl benzotriazole (1gm) in water and methanol (50ml) was refluxed at 80^0C 5-6 hours until it became a clear yellowish colour solution. This volume was reduced to 5ml and treated with methanol. The resulting brown crystals were collected and washed well with ethanol and acetone. The analytical data are given in Table 4.7.

(iv) Preparation of the Coordination Compound [Pt(5-Nitro-BZT_R)$_2Cl_2$] :

A mixture of $PtCl_2$ (500mg) and ligand 5-nitro benzotriazole (1gm) in water and methanol (50ml) was refluxed at 80^0C 5-6 hours until it became a clear white colour solution. This volume was reduced to 5ml and treated with methanol. The resulting white crystals were collected and washed well with ethanol and acetone. The analytical data are given in Table 4.7.

The general reaction for the preparation of coordination compounds of platinum is as follows :

$$[Pt\,(Cl)_2] + 2L \xrightarrow[H_2O]{CH_3OH} [Pt\,(L)_2(Cl)_2]$$

where L = 1,2,4-Triazole, 3-Amino-1,2,4-triazole, 5-Methyl benzotriazole and 5-Nitro benzotriazole.

(c) Analysis of the Constituents Elements :

(i) Carbon, Hydrogen, Nitrogen and Sulphur present in the investigated complexes were estimated micro-analytically.

(ii) Estimation of Pt :

For the estimation of platinum as ammonium chloroplatinate, dissolved the compound in 5ml of concentrated hydrochloric acid and 20ml of hot water, and then add gradually an equal bulk of half-saturated ammonium chloride solution. Allow to stand for 8 hours, filtered off the precipitate, wash it with ammonium chloride solution, and finally twice with cold water. Transfered the filtered paper and precipitate to a Main-Smith Crucible, heat extremely slowly at first, and ultimately raise to a bright red heat. Repeated heating, cooling and weighting were carried out until weight obtained constant.

(d) Physical Methods :

(i) Molecular Weight Determination :

Molecular weight determination of the synthesized complexes was made by Rast's method.

(ii) Magnetic Susceptibility Measurement :

The magnetic susceptibility measuerments were made at room temperature by the Gouy Method. A magnetic field strength of 8500 guass was employed. The apparatus was calibrated using cobalt mercury thiocynate $Hg[Co(NCS)_4]$. The diamagnetic corrections were computed using Pascal's constant **[45,46]**. For calculations of effective magnetic moment following equation has been used.

Effective magnetic moment (μ eff) = 2.84 $(X^{corr}_m T)^{½}$, where T = temperature in absolute scale and X_m = corrected molar susceptibility.

(iii) Conductance Measurement :

Conductance was measured in analytical grade methanol using dip type cell with the help of a Philips Conductivity Bridge.

(iv) Infrared Sperctroscopy :

Infrared spectra (4000-600 cm^{-1}) of the uncoordinated ligands and of the complexes were recorded as Nujol Mulls supported between sodium chloride platex (rock salt regions) on a Perkin Elmer Spectrum RXI Spectrometer.

(v) ^{1}HNMR Spectral Measurement :

^{1}HNMR Spectra of the synthesized compounds will be recorded on AC 300F Spectrometer (300MHz) using TMS as an internal standard.

(vi) Electron Spin Resonance Spectra :

Electron Spin resonance spectra of the complexes were recorded at room temperature on a Varium E-3 spectrometer using powdered sample at the microware frequency 9.53GHz. The 'g' values were calculated using the given equation.

$$g = \frac{714.44 \times \sqrt{(GHz)}}{H\ (G)}$$

where $\sqrt{}$(GHz) = microwave frequency in GHz at which sample operated, and H(G) = field in Gauss for the sample.

4.3: Properties of the Complexes :

The analytical and physical data of the ligand and its metal complexes are given in Table no 4.7. The complexes are non-hygroscopic and stable at room temperature. The solubility of different complexes are given in Table no. 4.9. They are soluble in DMF and

DMSO, Slightly soluble is acetonitrile and insoluble in other organic solvent. The colour of different complexes are given in Table no. 4.8. They do not possess sharp melting points.

4.4 : Result and Discussion :

(a) Magnetic Measurement :

The magnetic values of synthesized complexes measured at room temperature. The magnetic moment values of all the complexes are zero, hence, they are diamagnetic. The square planar geometry of complexes are evident from their diamagnetic nature.

(c) Conductance Measurement :

The analytical and physical data of the ligand and its metal complexes are given in Table no 4.7. The values of molar conductance are in the range 0.052-0.058 $\Omega^{-1}cm^2mol^{-1}$ suggesting non-electrolyte nature of the synthesized complexes.

(d) Infrared Spectroscopy :

The triazoles are known to exhibit thiol-thione tautomeism and as such they exist in any one of the two forms in the complexes **[243-244]**.

The ligand 1,2,4-Triazole possesses three possible cyclic donor sites one cyclic secondary nitrogen (NH) and other two cyclic tertiary nitrogen (=N-). Further the secondary nitrogen atom is involved in coordination. Coordination through nitrogen of the NH group invariably results a negative shift in υN_{cyclic} (1375 cm^{-1}) by at least 62 cm^{-1} (1313 cm^{-1}). In the complex of 3-Amino-1,2,4-triazole studied here. The ligand possesses four possible

donor sites, one amino group, one cyclic secondary nitrogen (NH) and other two cyclic tertiary nitrogen (=N-). Further the amino group is involved in coordination. Coordination through nitrogen of the amino group invariably results a negative shift in υNH_2 (3105 cm^{-1}) by at least 64 cm^{-1} (3041 cm^{-1}). The IR frequency of tertiary and secondary nitrogen of the triazole ring are unchanged, thereby, suggesting the cyclic nitrogen of this ligand do not participate in coordination. In the complex of 5-Methyl benzotriazole and 5-Nitro benzotriazole possess three possible cyclic donor sites, one cyclic secondary nitrogen (NH) and other two cyclic tertiary nitrogen (=N-). Further the secondary nitrogen atom is involved in coordination. Coordination through nitrogen of the NH group invariably results a negative shift in υN_{cyclic} (1375 cm^{-1}) by at least 30-70 cm^{-1} (1315 cm^{-1}, 1321 cm^{-1}). The formation of co-ordinate bond between the ligand and metal ion results in lowering the frequency (30-70 cm^{-1}) than the free ligand. This lowering in frequency has been attributed to the weakening of N-H bond, resulting from the drainage of electron density from the nitrogen on its coordination to the metal ion.

In addition, all the metal complexes show non-ligand bands in the far-IR region at 550-500 (M-N) and 450-400 cm^{-1} (M-O) **[245-47]**.

(d) Electron Spin Resonance Spectra :

The electron spin resonance data for the synthesized complexes under this investigation are given in Table 4.11.

The recorded 'g' values in the range 1.982-1.988 are constant. The electronic spectral bands of the complexes (Table 4.12) were assigned according to the literature **[47,48]**.

The molecular orbital approach was used to explain the structure of square-planar complexes of the d^8 elements. The metal orbitals involved in σ-bonding in square–planer complexes are the nd_{z2}, nd_{x2-y2} (n+l)s, (n+1)P_x and (n+1)P_y.' Nevertheless, judging from the values of the overlap integrals, ndx^2-y^2 (n=1)s, (n+1)P_x and (n+1)P_y account for most of the σ-bonds, and ndz^2 makes only a minor contribution. The most important π–molecular orbital and a combination of π–orbitals of the ligands.

The correlation of the bands observed in the electronic spectra for the studied complexes with those of $[M(CN)_4]^{2-}$ [M = pt^{11}] prompted us to assume the following assignments (Table 4.12) $^1A_{1g}\rightarrow{}^1A_{2g}[b_{2g}(\pi^*)\rightarrow b_{1g}(\sigma^*)]$, (d-d); $^1A_{1g}\rightarrow{}^1B_{1g}[b_{2g}(\pi^*)\rightarrow a_{1g}(\sigma^*)]$, (d-d); $^1A_{1g}\rightarrow{}^1E_g[e_g(\pi^*)\rightarrow b_{1g}(\sigma^*)]$, (d-d);$^1A_{1g}\rightarrow{}^1B_{1u}[b_{2g}(\pi^*)\rightarrow a_{2u}(\pi^*)]$, (C.T); $^1A_{1g}\rightarrow{}^1E_u[e_g(\pi^*)\rightarrow a_{2u}(\pi^*)]$, (C.T).

The relation between the bands in the present complexes and the described for the typical complexes $[M(CN)_4]^{2-}$ leads to the conclusion that all the new complexes have the same square-planar geometry.

(e) NMR Spectroscopy :

The PMR Spectra of the ligands exhibit signals at δ2.5 (m,CH_3), 7.0-8.0 (ArH), 10.2 (OH) and 12.6 (SH). This shows that the ligands exists in thiol form rather than thione form, which supports the IR obsevation and non-involvement of proton on the

sulphur in the reaction. The signals at δ10.2 in sprctra of the complexes, confirming that the hydroxy group has reacted with metal (II) moiety via deprotonation. The presence of a singlet at δ12.6 suggests that in the complexes the ligand retains thiol form. The signal due to azomethine proton in the complexes appears at δ9.0. The downfield shift observed indicates the deshielding effect due to the coordination of nitrogen to the central metal ion.

4.5 : Summary :

The mixed ligand complexes $[PtL_2Cl_2]$ where (L=1,2,4-triazole, 3-amino-1,2,4-triazole, 5-methyl benzotriazole, 5-nitro benzotriazole) have been prepared by the interaction of parent compound $[PtCl_2]$ with ligand. The complexes are characterized by elemental analysis, magnetic measurement, electron spin resonance and infrared spectram studies contain Pt (II) d^8 configuration. All the complexes are diamagnetic suggesting square planner geometry. It is observed that:

(i) The DMF and DMSO solution of the synthesized compounds are non-conducting.

(ii) All the complexes contain low spin d^8 configuration.

(iii) The reflectance spectra of the complexes display a shoulder at 340-430 nm, which is attributable to transition $A_{1g} \longrightarrow A_{2g}$

(iv) All the compounds are thermally stable upto 200^0C.

(v) All the complexes show anticancer activity.

(vi) Triazoles are known to exhibit thiol-thione tautomerism.

Table 4.1 : Isomer Ratios in the N-Methylation of Imidazoles[a]

Imidazole[b]	MeI	Me_2SO_4	CH_2N_2	Me_2SO_4/OH^-	Ag salt with MeI
4-Br [98]		: 34			
4-CN [90]				2.9 : 1	
4-CHO [100,573]		5-CHO-1-Me only			
4-CO_2Me [100]		1,5-isomer only			
4-Me[90d]	2 :1			2.2 : 1	
4-NO_2[96b,98,106]		1: 350	1 : 45	3 : 1	1,4-isomer only
4-Ph[96b,98]		5 : 1	1 : 2		
4-Ar-5-NO_2[103b]	Both isomers[c]	4-Ar-1-Me-5-NO_2			
4-Br-5-Me[96b]		5-Br-1,4-Me_2 only		2 : 3	
4-Br-5-NO_2[98]		4-Br-1-Me-5-NO_2 only			
4-Br-5-Ph[96b]		5-Br-1-Me-4-Ph only			
4-$CONH_2$-5-NO_2[106]					4-$CONH_2$-1-Me-5-NO_2 only
4-CHO-5-Me[100]		5-CHO-1,4-Me_2 only			
4-CO_2Me-5-NO_2[106]					5-CO_2Me-1-Me-4-NO_2 only
2-Me-4-NO_2[99]		1 : 50			
4-Me-5-NO_2[98,106]		233 : 1[d]			1,5-Me_2-4-NO_2 only

Table 4.1 : Continued

Imidazole[b]	MeI	Me_2SO_4	CH_2N_2	Me_2SO_4/OH	Ag salt with MeI
2-Me-4-Ph[218]				2.7 : 1[e]	
4-NO_2-5-$C_6H_4NO_2$(p)[98]		1-Me-5-NO_2-4-$C_6H_4NO_2$(p) only			
4-NO_2-5-CH:CHPh[106]					1-Me-5-NO_2-4-CH:CH
					Ph only
2,4-Br_2-5-Me[96b]		1 : 45[f]	1 : 10[f]	1 : 1	
2-Br-4-Me-NO_2[98]		2-Br-1,4-Me_2-5-NO_2 only			

(a) For di-substituted products the ratio given is that of 1,4-isomer : 1,5-isomer.

(b) In naming substituents the numbering used carries no implications for the tautomeric composition of the compound.

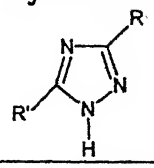
(c) MeI-K_2CO_3-acetone.

(d) In favour of 1,4-Me_2-5-NO_2.

(e) In favour of 1,2-Me_2-4-Ph.

(f)In favour of 2,5-Br_2-1,4-Me_2

Table 4.2 : *N*-Alkylation of 1,2,4-Triazoles



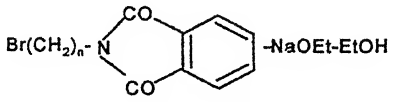
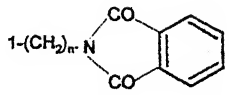
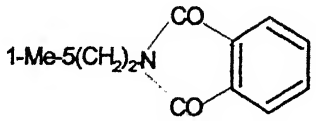
R	R′	Conditions	Products	Ref.
H	H	MeI-NaOMe-MeOH	1-Me	**225f,226a**
H	H	ErBr-NaOEt-EtOH	1-Et	**225f**
H	H	C_3H_5Br-NaOEt-EtOH	1-C_3H_5	**225f**
H	H	$PhCH_2Cl$-NaOEt-EtOH	Probably 1-CH_2Ph	**73c**
H	H	$BrCH_2CO_2Et$-Na OEt-EtOH	Probably-CH_2CO_2Et	**266**
H	H		Probably 	**227**
H	H	Na salt-benzene-Ph_3CCl	Probably 1-Ph_3C	**204a**
	H	MeI-NaOEt-EtOH	1-Me-3-and 	**228**
Me	H	Na salt-benzene-Ph_3CCl	Probably 1-Ph_3C	**204a**
Me	Me	MeI-NaOMe-MeOH	1,3,5-Me_3	**226a**
Me	Me	CH_2N_2-MeOH	1,3,5-Me_3	**226a**
Me	Me	EtI-NaOEt-EtOH	1-Et-3,5-Me_2(58%) +4-Et-3,5-Me(~1%?)	**226a**
Me	Me	CH_3CHN_2	1-Et-3,5-Me_2	**226a**
Me	Ph	MeI-NaOMe-MeOH	1,5-Me_2-3-Ph	**226b**
Me	Ph	CH_2N_2	1,3-Me_2-5-Ph and 1,5-Me_2-3-Ph in ratio 3.7:1	**226b**
Ph	H	MeI-NaOMe-MeOH	1-Me-5-Ph and 1-Me-3-Ph in ratio 1:2	**226b**
Ph	H	CH_2N_2	1-Me-5-Ph and 1-Me-3-Ph in ratio 1.6:1	**226b**
Ph	Ph	MeI-NaOMe-MeOH	1-Me-3,5-Ph_2	**226a,229**

Table 4.3 :

The Alkylation of Hydroxy-1,2,3-Triazoles[a]

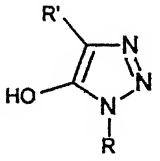
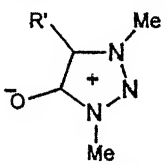
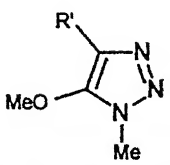
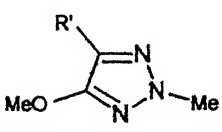
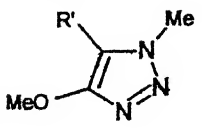
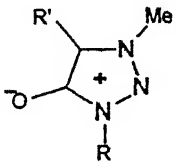
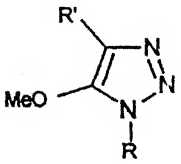
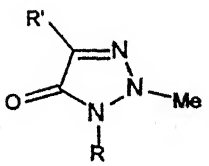
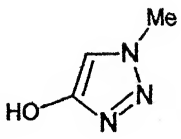
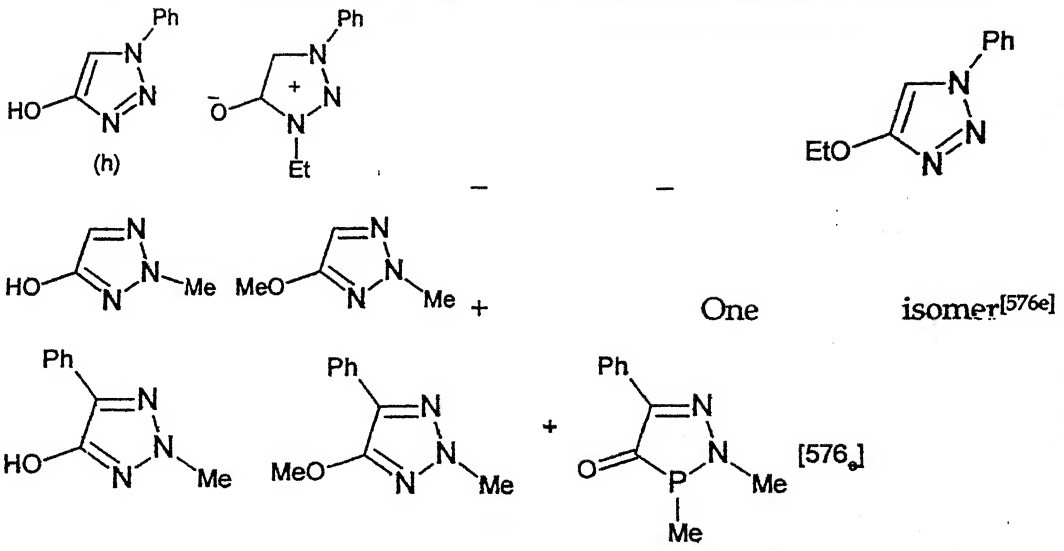
Starting Material		Products			
					
R	R′				
H	H	38%	2%	43%	5%
H	Ph	+	+	+[b]	+[b]
H	CO_2Et	27%	–	+[b]	+[b]
Me	H	+	+	–	–
					
CH_2Ph	H	+	+	+	–
CH_2Ph	H	100%[c]	–	–	–
Ph	Me[d]	62%	12%	25%	–
Me	Ph	+	42%	23%[e]	–
Me	CO_2Me	45%	54%	–	–
Ph	Ph[f]	30%	48%	12%	–
Ph	CO_2Et	–	[OEt compound]g	–	–
	+ (R=Me,R′=H)		–	–	+ (R′=H)

Table 4.3 : Continued



(a) Unless otherwise stated CH_2N_2+Et_2O-MeOH was used [120]. In this table + indicates that product was present, - that it was absent.

(b) Two products were obtained but definite assignments to these structures were not possible.

(c) Using MeI-NaOH-MeOH [120d].

(d) Esterifying conditions failed [170a].

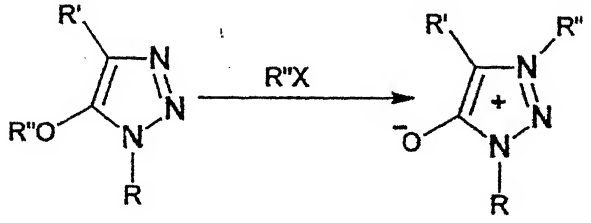
(e) Assumed structure.

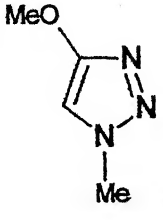
(f) Dimroth [170a], by treating 5-hydroxy-1,4-diphenyl-1*H*-1,2,3-triazole with MeI-NaOMe-MeOH or Me_2SO_4-NaOH, obtained a compound, m.p.126^{0}C, which regarded as 5-methoxy-1,4-diphenyl-1*H*-1,2,3-triazole. The true methoxy compound has m.p.86-7^{0}C [120c] and Dimroth's product remains unidentified. See also ref.[171].

(g) Using EtI-silver salt.

(h) Using Et_2SO_4-NaOH-EtOH [120d,172]

Table 4.4: Reactions of 1-Substituted 4- and 5-Acyloxy- and 5-Alkoxy-1,2,3-Triazoles with Alkyl Iodides

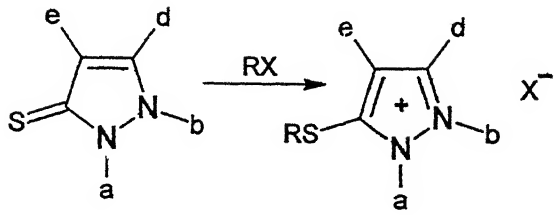


Quaternary salt not detected	R	R/	R//	R///X	Ref.
	Me	Ph	Me	MeI	**120c**
	Me	EtO_2C	Me	MeI	**120c**
	$PhCH_2$	H	Me	MeI	**120c**
	Ph	Me	Me	MeI	**120c**
Quaternary salt detected	Ph	H	Me	MeI	**120c**
	Ph	H	Me	EtI	**120c**
Quaternary salt isolated	Me	H	Me	MeI	**120a,b**[a]
	$PhCH_2$	H	PhCO	MeI	**120d**
				MeI	**120a,b**[a]
				ditto $PhCH_2I$	**120c**

(a) The quaternary salt very readily decomposed in solution ($CHCl_3$) to give the meso-ionic product.

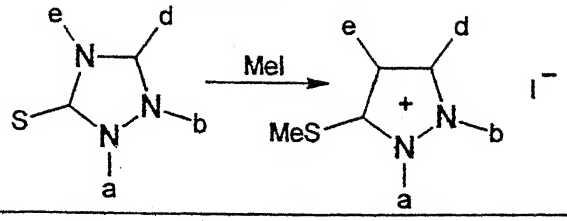
Table 4.5 :

Some Quaternary Salts Formed from Sulphur Analogues of Antipyrine

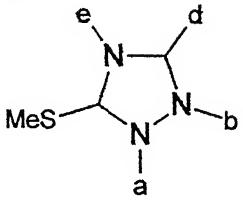


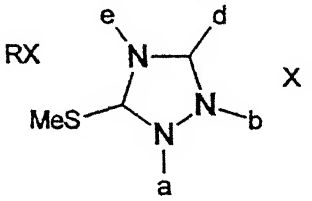
A	b	d	e	Ref.
Me	Me	Ph	H	**143d**
Me	Ph	Me	H	**143a**
Me	Ph	Me	Me	**143c**
Ph	Me	Me	H	**213b**
Ph	Me	Me	PhCO	**241h**

Table 4.6 :
Some Quaternary Salts Formed from Sulphur Derivatives of 1,2,4-Triazole[a]



A	b	d	e	Ref.
Ph		Me	Ph	**242c**
Ph		Me	Me	**181a**
Me	Ph	Me		**181a**
	Me	Me	Me	**242c,d**
	Ph	Me	Me	**181a**





A	b	d	e	RX	a	b	d	e	Ref.
Ph		Me		MeO_3S-C_7H_7	Ph		Me	Me	**181a**[b]
Ph		MeS		Could not be quaternised					**181a**
	Ph	Me		MeI or MeO_3S-C_7H_7		Ph	Me	Me	**181a**[c]
		Ph	Me	Me_2SO_4	Me		Ph	Me	**179**[d]
		Me	Me	MeX		Me	Me	Me	**181a,190c**[e]
		Me	Et	MeX		Me	Me	Et	**181a,190c**[e]
		Me	Ph	MeX		Me	Me	Ph	**181a,190c**[e]

(a) Skeletal structures are used to facilitate tabulation.

(b) The C-methyl group was unreactive.

(c) The C-methyl group was reactive but the MeS group was not.

(d) The MeS group was reactive.

(e) C-methyl was reacctive,

Table 4.7 :

Analytical and Electronic Spectral Data of Complexes of Pt(II)

Compound	Found(Calc.)%						Mol. Wt.
	M	C	H	N	S/O	Cl	Found(Calc.)
	47.87	11.32	1.10	20.05	-	17.13	403.90
$[Pt(C_2H_3N_3)_2Cl_2]$	(48.27)	(11.89)	(1.50)	(20.80)	-	(17.55)	(404.21)
	44.46	10.68	1.32	25.23	-	15.89	4334.82
$[Pt(C_2H_4N_4)_2Cl_2]$	(44.93)	(11.07)	(1.86)	(25.81)	-	(16.33)	(434.24)
	36.23	31.03	2.02	15.30	-	13.00	531.00
$[Pt(C_7H_7N_3)_2Cl_2]$	(36.65)	(31.59)	(2.65)	(15.79)	-	(13.32)	(531.39)
	32.04	23.80	1.00	18.40	10.20	11.45	594.00
$[Pt(C_6H_4N_4O_2)_2Cl_2]$	(32.83)	(24.26)	(1.36)	(18.86)	(10.77)	(11.93)	(594.33)

Table 4.8 :

Colour and % Yield of the Complexes

S.No.	Compound	Colour	%Yield
1.	$[Pt(1,2,4\text{–}TAZ)_2Cl_2]$	Gray	72
2.	$[Pt(3\text{–amino–}1,2,4\text{–}TAZ)_2Cl_2]$	Gray-white	64
3.	$[Pt(5\text{–methyl–}BZT_R)_2Cl_2]$	Brown	70
4.	$[Pt(5\text{–nitro–}BZT_R)_2Cl_2]$	White	68

Table 4.9 :

Solubilities of the Complexes in Different Solvents

S.No. Compound	DMF	DMSO	EtOH	MeOH	Acetonitrile	Ethylacetate
1. $[Pt(1,2,4\text{–}TAZ)_2Cl_2]$	soluble	soluble	insoluble	insoluble	sparingly soluble	insoluble
2. $[Pt(3\text{–amino–}1,2,4\text{–}TAZ)_2Cl_2]$	soluble	soluble	insoluble	insoluble	sparingly soluble	insoluble
3. $[Pt(5\text{–methyl–}BZT_R)_2Cl_2]$	soluble	soluble	insoluble	insoluble	sparingly soluble	insoluble
4. $[Pt(5\text{–nitro–}BZT_R)_2Cl_2]$	soluble	soluble	insoluble	insoluble	sparingly soluble	insoluble

Table 4.10 :

Important IR Spectral Bands and Their Assignments

S. No.	Compound	$\nu(NH_2)$	$\nu(N)_{cyclic}$	C-NO_2
1.	$[Pt(1,2,4\text{–}TAZ)_2Cl_2]$	-	1313 cm^{-1}	-
2.	$[Pt(3\text{–amino–}1,2,4\text{–}TAZ)_2Cl_2]$	3041cm^{-1}	1375 cm^{-1} (unchanged)	-
3.	$[Pt(5\text{–methyl–}BZT)_2Cl_2]$	-	1315 cm^{-1}	-
4.	[Pt(5–nitro–BZT)	-	1321cm^{-1}	2150cm^{-1}

Table 4.11 :

Electronic Spectral Data of the Complexes

$[PtCl_2(L)_2]$	21702	$^1A_{1g} \rightarrow {}^1A_{2g}[b_{2g}(\pi^*) \rightarrow b_{1g}(\sigma^*)]$
	265004	$^1A_{1g} \rightarrow {}^1B_{1g}\ [b_{2g}(\pi^*) \rightarrow a_{1g}(\sigma^*)]$
	304003	$^1A_{1g} \rightarrow {}^1E_g[e_g(\pi^*) \rightarrow\ b_{1g}(\ \sigma^*)]$

Table 4.12 :

Electronic Spectra of ML_2Cl_2 Chelates

Transition	**$PtCl_2L_2$**
d-d	
$^1A_g \rightarrow {}^3B_{1g}(x^2\text{-}y^2 \rightarrow xy)$	
$^1A_{1g} \rightarrow {}^1B_{1g}(x^2\text{-}y^2 \rightarrow xy)$	18, 181 (3.46)
$^1A_g \rightarrow {}^3B_{3g}(xz \rightarrow xy)$	
$^1A_g \rightarrow {}^1B_{3g}(xz \rightarrow xy)$	17, 482 (3.59)
M→L charge transf	
$^1A_{1g} \rightarrow {}^1B_{2u}[xz \rightarrow L(\pi^*)]$	17, 479 (3.55)
$^1A_g \rightarrow {}^1B_{3u}[yz \rightarrow L(\pi^*)]$	28, 005 (3.90)
M→L charge transfer	
$^1A_{1g} \rightarrow {}^1B_{2u}\ {}^1B_{3u}[L(\pi) \rightarrow xy]$	35, 78 (4.10)
$^1A_g \rightarrow {}^1B_{2u}\ {}^1B_{3u}[L(\sigma) \rightarrow xy]$	41, 838 (4.50)
L→L*	
$^1A_g \rightarrow {}^1B_{2u}$	29, 938 (4.04)
$^1A_g \rightarrow {}^1B_{1u}$	38, 171 (4.27)

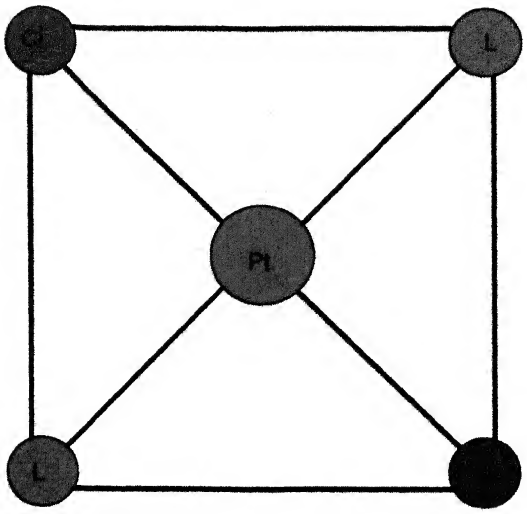


Fig .4.1 : Proposed square planar structure of $[Pt(L)_2Cl_2]$ (where L= 1,2,4-Triazole, 3-Amino1,2,4-Triazole, 5-Methyl benzotriazole, 5-Nitro benzotriazole)

Fig 4.2 : 1,2,4 – Triazole

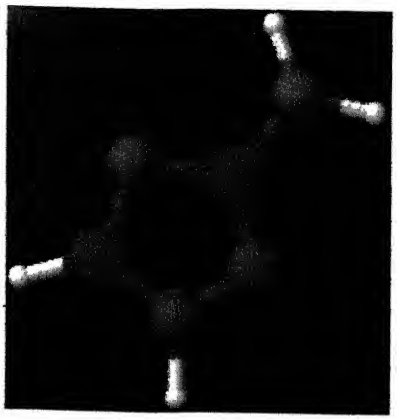


Fig 4.3 : 3-Amimo 1,2,4 – triazole

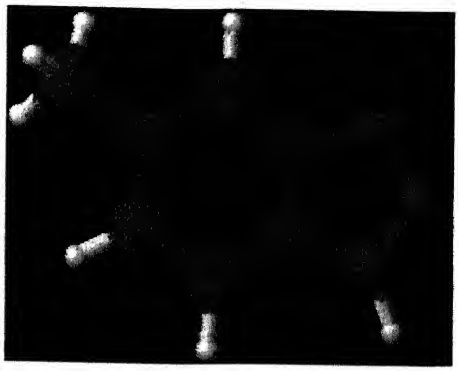


Fig 4.4 : 5-Methyl benzotriazole

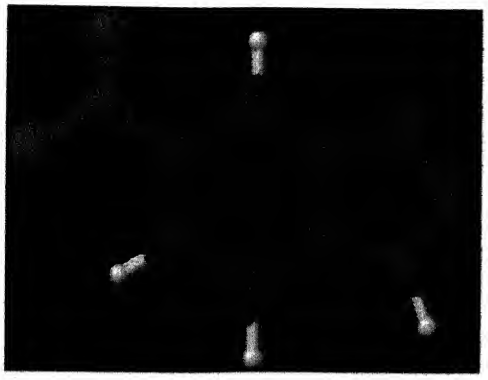
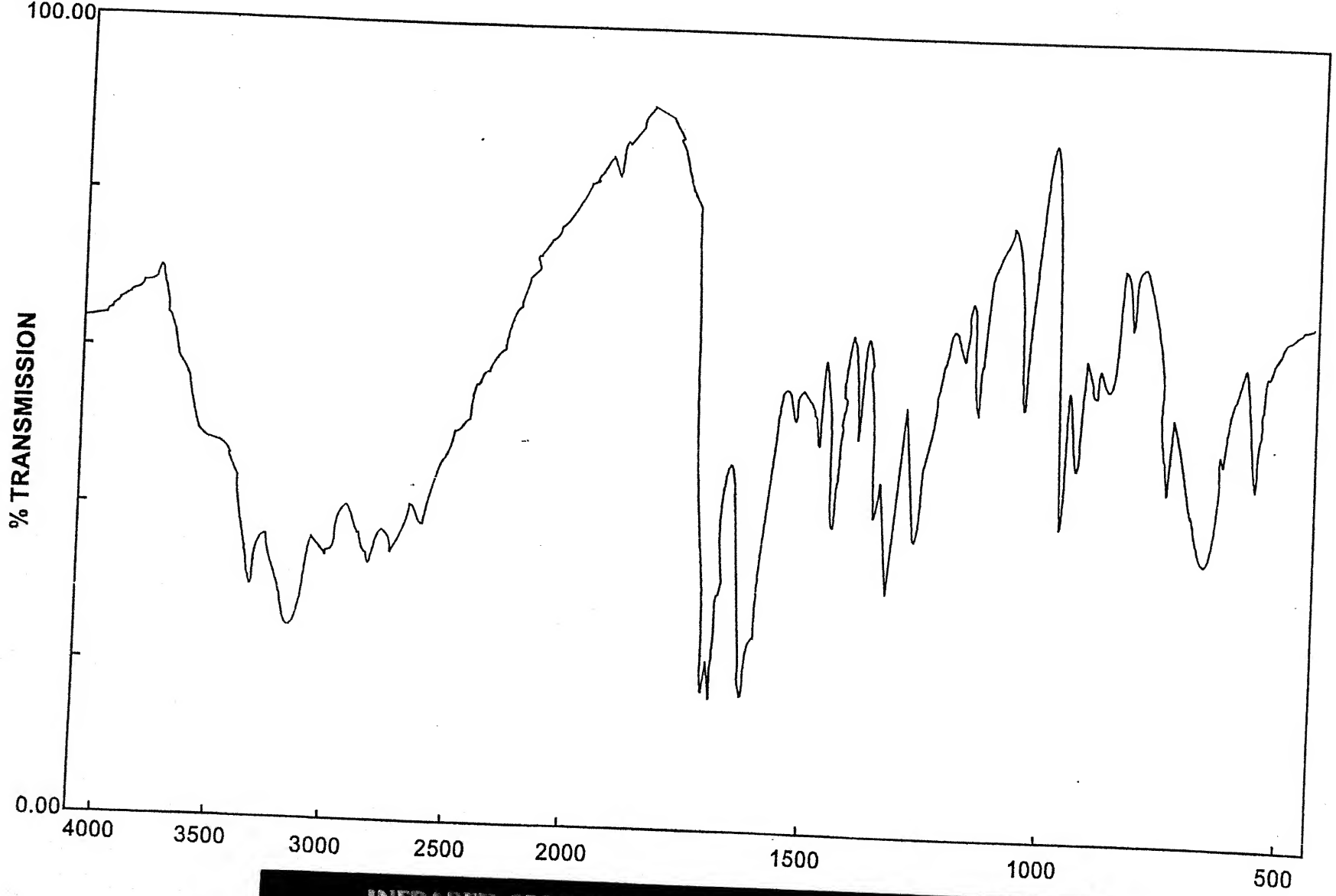
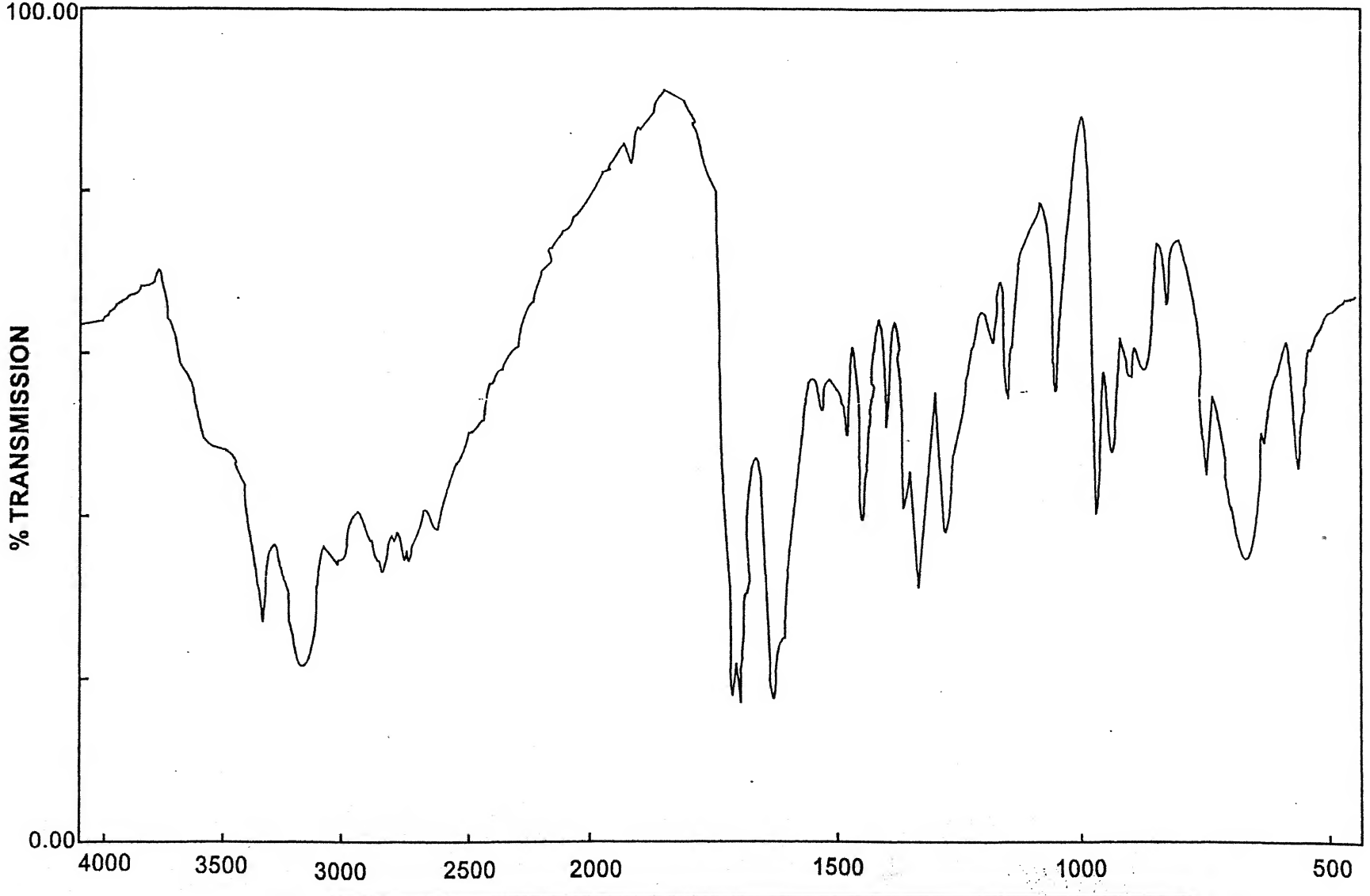


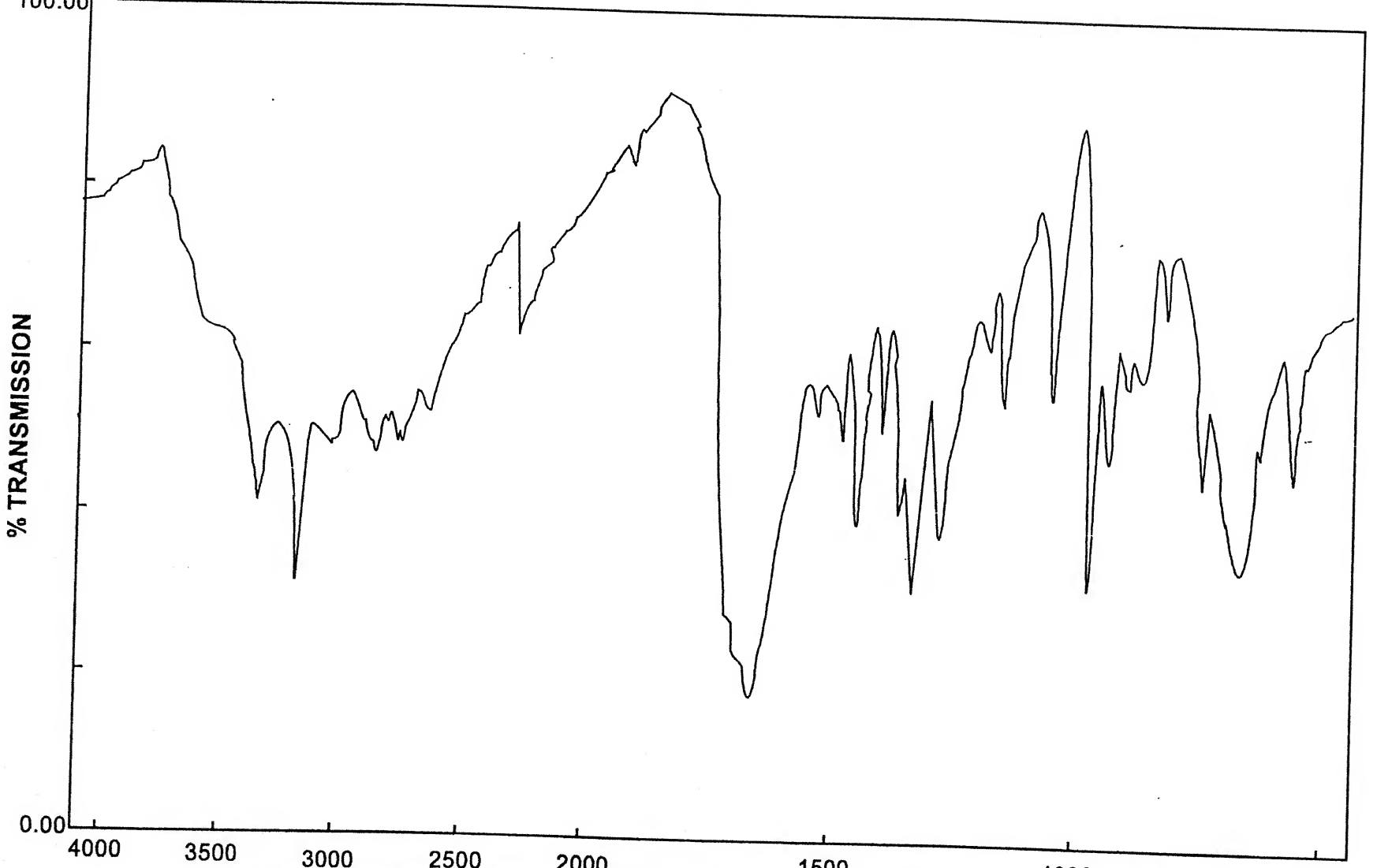
Fig 4.5 : 5-Nitro benzotriazole



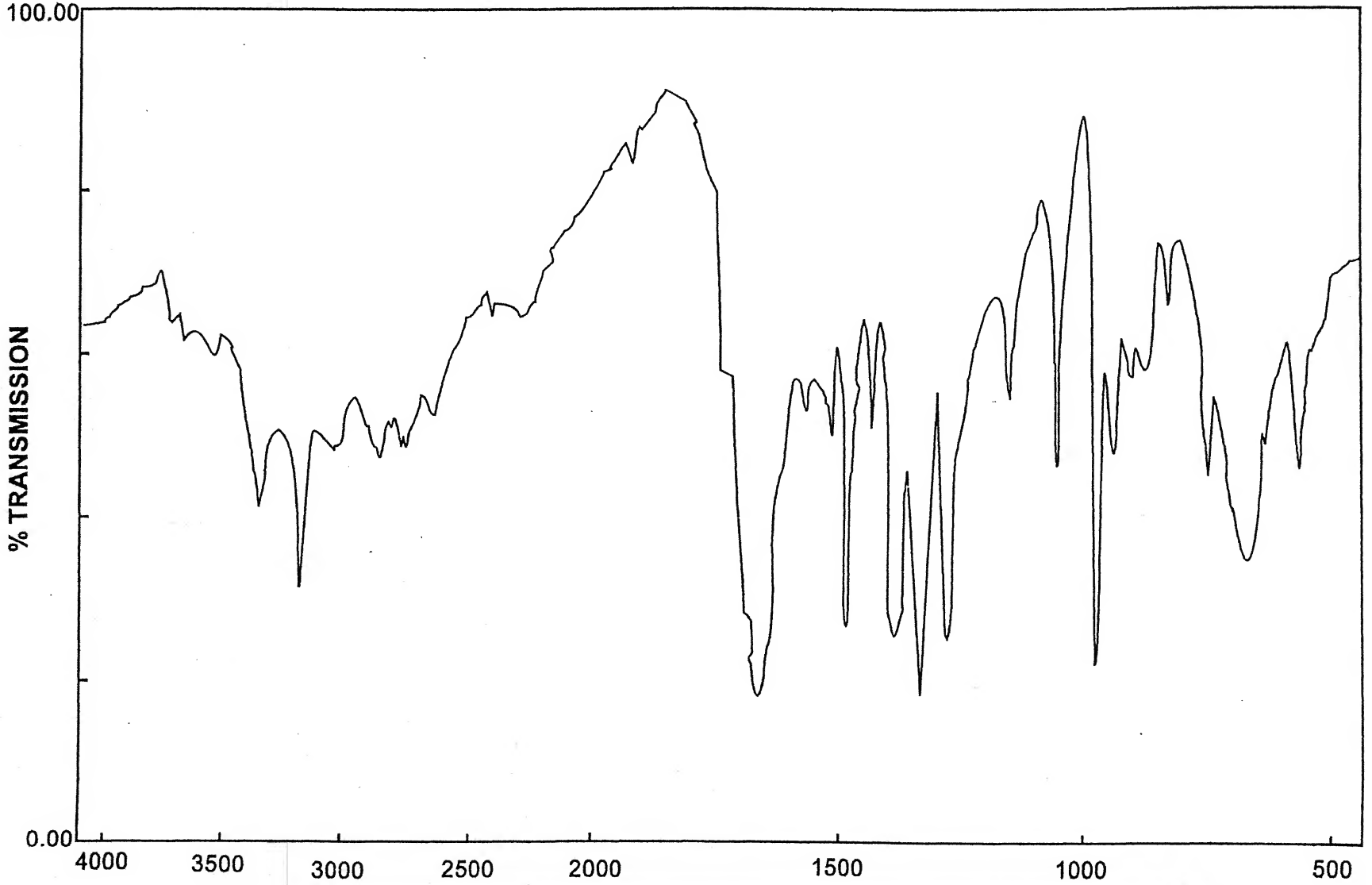
INFRARED SPECTRUM OF $[P(1,2,4\text{-}TAZ)_2Cl_4]$



INFRARED SPECTRUM OF $[Pt(3\text{-Amino-}1,2,4\text{-TAZ})_2Cl_2]$



INFRARED SPECTRUM OF [Pt(5-Methyl-BZT$_{R}$)$_2$Cl$_2$]



INFRARED SPECTRUM OF [Pt(5-Nitro-BZT$_{R_2}$)Cl$_2$]

CHAPTER-V

CHAPTER-V

5.1 : Clinical Studies With cis-$[PtCl_2(NH_3)_2]$:

Since the commencement of phase 1 clinical trials in 1972 under the auspices of the U.S. National Cancer Institute, cis-$[PtCl_2(NH_3)_2]$ (cisplatin) has shown significant activity against a range of tumor types, particularly those of the genitourinary region. Extensive data have been compiled for the treatment of testicular and ovarian cancer patients. The compound is now recommended as first–line chemotherapy for these tumors, particularly in combination with other approved chemotherapeutic agents. Other tumors have shown notable responses, but the data are not yet extensive enough for official approval; these include bladder, head and neck, cervix, prostate, and lung tumors.

Human studies with radioactive platinum confirmed animal work and showed high uptake in the kidney, liver and intestine **[266-696]**. Evidence from brain scans and brain tissue samples suggested poor penetration of the drug in to the central nervous system **[269,270]**. After initial injection, Pt was rapidly cleared from the blood and more than 90% of that remaining in the post-distribution phase (after approximately 30 min) was protein-bound **[271,272]**.

Cis-$[PtCl_2(NH_3)_2]$ is excreted primarily in the urine but only 30-50% of the injected dose is excreted in the first 5 days **[271]**; the remaining platinum, much of which is in the liver, is only removed slowly. Nephrotoxicity is the major dose-limiting toxicity for the drug, but this has been largely overcome by the use of pre-hydration with diuresis and infusion techniques. Whereas, renal toxicity was originally significant with doses around 50 $mg.M^{-2*}$, doses around 120 $mg.M^{-2}$ can

be safely given, using these techniques. The ability of the drug to act synergically in combination with other established chemotherapeutic drugs has led to the use of regimens involving low non-nephrotoxic doses. Bone marrow toxicity occurs, largely associated with a reduction in circulating white blood cells, although this is lower than that for most other anti-tumor drugs. Other side effects include nausea, vomiting, and high frequency hearing loss (ototoxicity). Peripheral neuropathy has been observed on repeated treatment.

In December 1978 cis-[$PtCl_2(NH_3)_2$] received U.S. Food and Drug Administration approval as an anticancer drug for testicular and ovarian cancers. The formulated drug containing sodium chloride and mannitol is marketed under the name of platinol. British approval of the drug came in March 1979; it is known in the U.K. as Neoplatin. License applications are pending in many other countries.

5.2 : Mechanism of Antitumor Action of Metal Complexes:

Filamentous growth in bacteria is indicative of the ability of an agent to react with DNA, giving selective inhibition of DNA synthesis but no accompanying effect on other biosynthetic pathways. Induction of bacteriophage from lysogenic bacteria and the mutogenicity of some active complexes are also important evidence for direct DNA attack **[273, 274, 288]**.

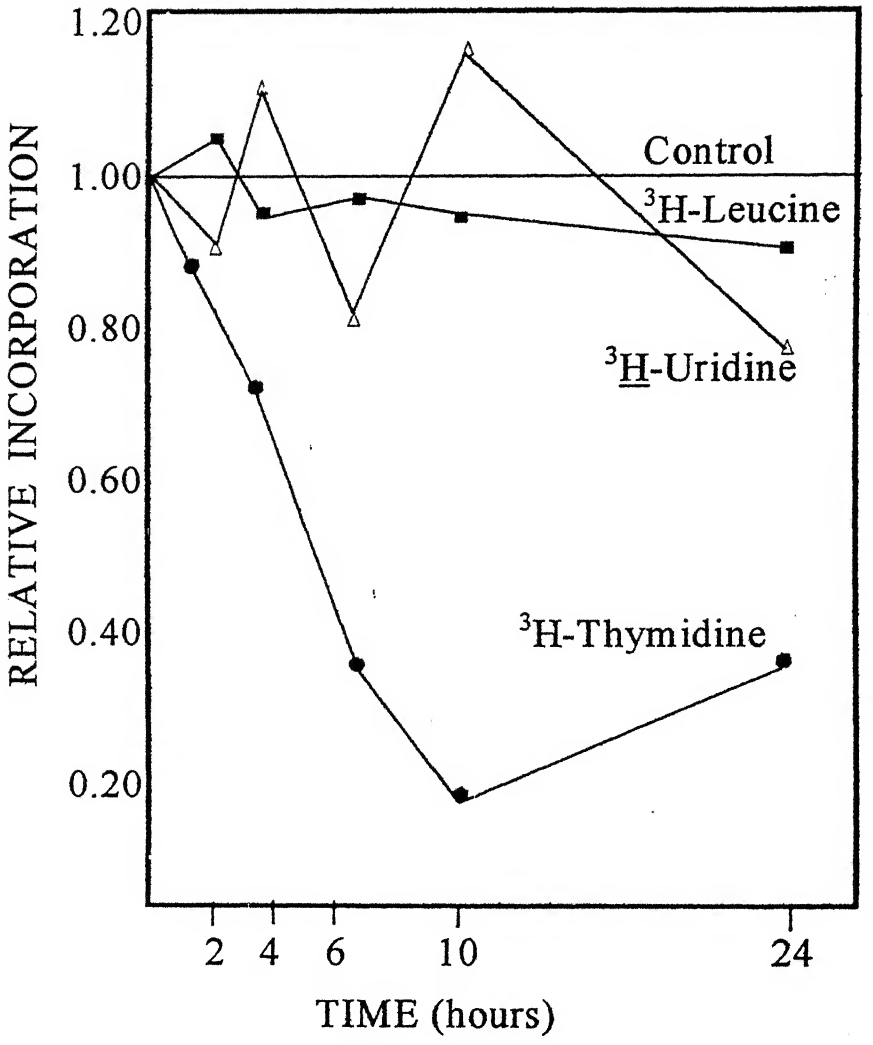
Biochemical studies on cells in culture have shown that cis-[$PtCl_2(NH_3)_2$] selectively and persistently inhibits the rate of DNA synthesis, as compared to RNA and protein synthesis **[275-78]** (Fig.5.1). It is postulated that the primary chemical lesion is in the DNA, inhibiting

it as a template for replication but not affecting transcription or translation.

Studies on cis and trans isomer of $[PtCl_2(NH_3)_2]$ on cells in vitro showed that trans binds to cell macromolecules as effectively as cis **[277]**. There are more platinum moieties bound per molecule of DNA than to either RNA or protein. Interstrand cross-linking has been demonstrated to occur for Pt compounds, but to a much lesser extent than for alkylating agents. The balance of evidence highly favours the proposal that this form of binding to DNA is not an important cytotoxic event **[278]**. Linking between bases on the same strand has been proposed **[279]** and some evidence for this has been obtained, particularly from work on the inactivation of bacteriophage **[280]**.

Guanine, adenine, and cytosine react with both $[PtCl_2(NH_3)_2]$ isomers, the rate being fastest for guanine **[281-83]**; the N-7 sites of adenine and guanine and N-3 sites of cytidine are favoured. A very slow reaction occurs with thymine. Studies on DNAs with varying GC-AT ratios suggest guanosine as a major reaction site **[284,285]**.

Intrastrand linking between neighbouring guanine bases has been proposed **[286]**. Chelation between the N-7 and 0-6 on the same guanine has been claimed for the cis and not the trans isomer **[287]** from ESCA studies and is a current point of controversy (Figure 5.1).



Fg. 5.1 Selective inhibition of DNA synthesis in human AV_3 cells grown in tissue culture with exposure to 5 μM cis-$[PtCl_2(NH_3)_2]$ **[250].**

It seems that only a proportion of the lesions caused by cis-$[PtCl_2(NH_3)_2]$ can be recognized by a DNA excision repair process although certain mammalian cells have effective postreplication repair systems. Inability to synthesize past some Pt lesions eventually leads to cell death **[272]**. An interesting question which remain unanswered is whether the many different active platinum compounds now reported produce the same type of lesion. Are all platinations equal, or do some compounds give a higher proportion of a more lethal lesion, leading to

less Pt on the DNA at the therapeutic dose? This could give less overall toxicity.

5.3 : Antitumor Studies :

The property of inhibiting cell division, but not cell growth, suggested that these compounds might have antitumor properties; this was emphasized by the fact that known antitumor agents caused elongation and lysis in lysogenic bacteria. Initially four compounds–cis-$[PtCl_4(NH_3)_4]$, cis-$[PtCl_2(NH_3)_2]$, $[PtCl_4(en)]$ and $[PtCl_2(en)]$—were tested against Sarcoma 180 in the ICR strain of mice and were found to be effective in inhibiting the tumor growth **[248]**. As had been predicted by the bacteriological results, neither of the trans isomer showed any appreciable activity. The two cis compounds were submitted to the U.S. National Cancer Institute and screened against L1210 leukemia in mice **[248]**. The compounds showed potent antitumor activity and effected several cures with single injections at the therapeutic dose of 8 mg. kg^{-1} of body weight. Rosenberg and Van Camp went on to show that cis-$[PtCl_2(NH_3)_2]$ was capable of regressing large solid Sacroma-180 tumors (8 days old) in Swiss white mice **[251]**. Cis-$[PtCl_2(NH_3)_2]$ appeared to be the more potent of the original compounds and has since been tested against many transplanted animal tumors and has proved to have a wide spectrum activity. It has been under extensive toxicological and clinical trials leading to recent governmental approval for certain human tumor.

Since the original discovery of anticancer effects of Pt complexes, studies have been undertaken to determine what relationship exists between chemical structure and antitumor activity with a view to finding

more active, less toxic drugs **[252-58]**. This work has been discussed in several reviews and a summary account is given below **[249,259]**.

Platinum complexes of the type $[PtX_2A_2]$ (X_2 = two monodentate anionic ligands or one bidentate anionic ligand; A_2 = two monodentate amine ligands or one bidentate amine ligand) have attracted the most attention while limited data on other systems have been reported. It has been established that where activity exists, it is only found in neutral species and in cis rather than trans isomers **[252,258]**. The X and A ligands have been systematically varied and the chemistry of active compounds has been investigated, particularly in kinetic terms **[259]**.

Several compounds have been identified which show better or comparable activity against several animal tumors, to cis-$[PtCl_2(NH_3)_2]$. Some of these compounds have the advantage, from the viewpoint of mode of administration, of relatively high water solubilities although these often require higher doses to achieve maximum effects.

(a) Structure-Activity Studies :

Initial studies concentrated on variation of the anionic ligands (X) in species of the type cis-$[PtX_2(NH_3)_2]$, using the solid Sarcoma-180 tumor in Swiss white mice **[252]**. The results indicated that in the amine system, labile groups such as NO^-_3 and H_2O give rise to highly toxic species, while strongly bound ligands such as SCN^- and NO^-_2 formed inactive complexes. Activity was found with monodentate ligands of intermediate lability (e.g., Cl^- ,Br^-). Since this early work, several groups of researchers have reported on the complex nature of cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ solutions which tend to polymerize on standing via the formation of hydroxo

bridges **[260-61,58]**; the monomer and oligomers have widely different toxicities, and activities with the dimer being particularly toxic.

A major outcome of this phase of the work was the activity of complexes with the chelating dicarboxylate ligands, oxalate, malonate, and substituted malonates. Further testing against the ADJ/PC6A solid plasma cell tumor in BALB/C mice at the Chester Beatty Institute confirmed these results (Table 5.1). Subsequent synthesis and testing on other amine systems have shown that complexes with these ligands usually have comparable or superior activity to those containing chloride groups.

Tobe and Connors et al. studied the effect of varying the amine ligands (A_2) in species of the type cis-[$PtCl_2A_2$] and found them to have a primary effect on the antitumor properties **[258]**. The ADJ/PC6A tumor appears to be very sensitive to platinum compounds and relatively minor structural changes can lead to major changes in the therapeutic index (TI). Most of the changes in TI are associated with toxicity rather than potency. Heterocyclic and alicyclic amines (Table 5.2), and straight and branched chain alkylamines all give compounds with appreciable activity and selectivity. Unfortunately, the highest TIs were associated with compounds of extremely low aqueous solubility (injected intraperitoneally as a suspension in arachis oil), which means that they are probably acting as slow release systems, thus making a true comparison of toxicity impossible. Chelating amines such as 1,2-diaminocyclohexane and o-phenylenediamine were also

effective. Results on the plasma cell tumor indicated that toxicity may not be so closely related to activity as to prevent the existence of active complexes with relatively low toxicities. The S180 tumor was much less sensitive to variations in amine ligands although primary alkylamines showed more activity than secondary **[252]**.

Results on the L1210 tumor in BDF1mice **[259,262]** have confirmed the activity of straight- and branched-chain alkylamines and alicyclic amines, particularly at the C_3-C_4 level (Table 5.3). The most impressive results were obtained on a daily 1-9 dose schedule. Preliminary estimates of nephro- and myelotoxicity on mice by measurement of blood urea nitrogen (BUN) levels in urine and white blood cells (WBC) in blood indicate that some compounds may be less toxic in both respects than cis-$[PtCl_2(NH_3)_2]$.

Some malonato and substituted malonato complexes have also shown promise in this system (Table 5.3). Tobe and Connors et al. went on to exploit the (IV) oxidation state of platinum in order to synthesize more water-soluble compounds. Trans dihydroxo Pt (IV) species were synthesized and the hydrophilic properties of the OH^- ligands gave improved aqueous solubility while maintaining the activity in some cases **[258]**. In general the order of activity was

$$[Pt(II)Cl_2A_2] > [Pt(IV)Cl_4A_2] \sim [Pt(IV)Cl_2A_2(OH)_2]$$

Although variation in lipid solubility were noticed, these did not correlate with antitumor effect.

Present efforts appear to be concentrated on a comprehensive study of the more effective amine systems, reported by Tobe and Connors et al. For chloro complexes, with a range of different leaving groups in an attempt to optimize activity and solubility **[259]**. The ligands under investigation include bi- and monodentate carboxylates and O-bonded anionic groups such as sulphate and nitrate and phosphate.

Gale **[256]** and their co-workers have reported similar studies which have concentrated on the 1,2-diamino–cyclohexane system. Little data is available but initial results indicate that at least some compounds will match or improve upon the activity shown in the chloro species (Table 5.4).

Several of the promising compounds have been subjected to animal testing against a wide variety of transplanted tumors, mainly via the U.S. National Cancer Institute. The compounds $[Pt(Etmal)(NH_3)_2]$, $[Pt(CBDCA)(NH_3)_2]$ (CBDCA=1-cyclobutanedi carboxylate), and $cis[PtCl_2(OH)_2$ (isopropyl)] show good activity against most system; a selection of results for the latter is shown in Table 5.5 **[259]**. All of these compounds have relatively high aqueous solubilities.

(b) Reactivity of Antitumor–Active Species :

The chemistry of platinum (II) amine species of the type $[PtX_2A_2]$ is dominated by the high affinity of NH_3 for the Pt (II) centre. Affinities for common ligands vary **[263]**:

$$CN^- > NH_3 \sim OH^- > I^- > SCN^- > Br^- > Cl^- > F^- \sim H_2O$$

The strength of the Pt-N bond tends to override the trans effect factors, which often control the substitution kinetics of Pt (II) complexes (along with smaller cis effects). Organic amines, especially the simpler alkyl– and alicyclic amines are expected to be have similarly to NH_3. Thus chemical studies on both isomers of $[PtX_2(NH_3)_2]$ have clearly shown that X are the reactive or leaving groups, while the NH_3 ligands are relatively inert **[264,265]**. The order of leaving ability has been established for the reaction **[265]**

$$[Pt(dien)X]^+ + py = [Pt(dien)py]^{2+} + X^-$$

where the order of decreasing rate constant is

$$X = NO_3^- > H_2O > Cl^- > Br^- > I^- > SCN^- > NO^-_2$$

The spread in rates for the reaction is about 10^6, showing that the leaving group X and the consequent breaking of the Pt-X bond have a substantial effect on the reaction site. The testing results against the S180 system reflected this order, with strongly bound (poor leaving) ligands giving rise to inactive species, while reactive aquo ligands were very toxic. However, aquo species from bulkier organic amines were far less toxic **[252]** and recent studies have shown that X ligands (such as sulphate, which is expected to be very reactive) can give rise to active species. Much of the aquo species toxicity appears to be due to polymerization by formation of hydroxo bridges, which seems to be a slow process for some of organic amine systems.

The amine ligands (A) have a primary effect on the antitumor properties, while they are expected to have only a secondary effect on reactivity via different steric, electronic, and basic properties.

Aqueous solutions of organic amine complexes containing various leaving ligands have been examined by conductivity and ultraviolet/visible spectroscopy techniques in order to compare their reactivities against a common incoming group **[259]**. The results may be summarized as follows. Variation in the (A) group has little effects on the solvolysis of the complexes by water and DMSO. The wide variations in activity do not correlate with reactivity and appear to be due to biophysical, rather then chemical factors.

Although the empirical rules concerning neutrality and a cis configuration still hold, we can now identify three classes of active compounds on a kinetic basis:

1. Reactive species, such as sulphato and nitrato complexes, which are rapidly hydrolyzed and also converted to chloro species in the presence of physiological levels of saline.
2. Species with intermediate reactivity towards water and chloride. Reactions of chloro complexes themselves are, of course, suppressed in the presence of chloride ion which serves to protect them in the serum. Compounds containing halogenoacetate ligands will undergo chloride replacement at an intermediate rate.
3. Bidentate carboxylate complexes are the only kinetically inert species to show activity so far. These are so non-reactive in comparison to other antitumor-active species that we have previously mentioned that an in vivo activation mechanism might be operating, possibly involving enzymes **[249]**.

Table 5.1 :

Screening data for Platinum(II) Malonate Derivatives Against the ADJ/PC6A Tumor (reported complex)

Malonato complexes	LD_{50} ($mg.kg^{-1}$)	ID_{90} ($mg.kg^{-1}$)	TI	Aqueous solubility(mM)
$[Pt(NH_3)_2mal]$	225	18.5	12.2	1.0
$[Pt(NH_3)_2Memal]$	112	4.5	24.9	7.0
$[Pt(NH_3)_2Etmal]$	132	12.0	11	160.0
$[Pt(NH_3)_2OHmal]$	150	4.9	30.6	-
$[Pt(NH_3)_2Benzmal]$	150	1.85	81.1	-
$[Pt(NH_3)_2(1,1\text{-}CBDCA)]$	180	14.5	12.4	50
[Pt(en)mal]	220	18.5	12	-
[Pt(en)Memal]	200	50	4	-
[Pt(en) Etmal]	450	49	9.2	-
$[Pt(MeNH_2)_2mal]$	670	56	12	–

Table 5.2 :

Screening Data for Alicyclic Amine Complexes Against the ADJ/PC6A Tumor (reported complex)

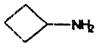
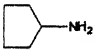
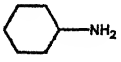
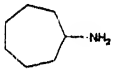
A	solvent	Dose range ($mg.kg^{-1}$)	Dose response	LD_{50} ($mg.kg^{-1}$)	ID_{90} ($mg.kg^{-1}$)	TI
ADJ/PC6A plasma cell tumor						
NH_3	A	0.1-40	+	13.0	1.6	8.1
CH_3NH_2	A		-	18.5	18.5	1.0
$ClC_2H_4NH_2$	A		+	45.0	17.5	2.6
	A	2.5-160	+	56.5	2.6	21.7
	A	3-200	+	141	10.8	13.1
	A		-	90	>90	<1.0
	A		-	18	>18	<1.0
	A	1-80	+	56.5	2.3	24.6
	A	6-750	+	90	2.9	31.0
	A	1-3200	+	565.6	2.4	235.7
	A	1-3200	+	>3200	12	>267
	A	5-625	+	>625	18	>35

Table 5.3 :

Screening Data for Platinum Complexes Against L1210 Tumor (10^6 Cells) in BDF_1 Mice (reported complex)

Complex	optium dose (mg kg^{-1})	Schedule (days)	Median survival (%T/C)	Therapeutic ratio (MTD/MED)	Toxicity	
					(BUN)	(WBC)
cis $[PtCl_2(CH_3NH_2)_2]$	16	1	129	1	+[a]	±[b]
	2	1-9	121	1		
cis$[PtCl_2(n\text{-}C_3H_7NH_2)_2]$	8	1	157	2	+	+
	8	1-9	157	2		
cis$[PtCl_2(i\text{-}C_3H_7NH_2)]_2$	32	1	171	1	+	±
	8	1-9	179	2		
cis$[PtCl_2(C_3H_5NH_2)_2]$	16	1	157	4	+	+
	8	1-9	164	2		
cis$[PtCl_2(OH)_2(i\text{-}C_3H_7NH_2)_2]$[c]	32	1	171	2	+	±
	16	1-9	207	2		
cis$[PtCl_2(i\text{-}C_4H_9NH_2)_2]$	64	1	171	4	+	±
	16	1-9	193	8		
cis$[PtCl_2(t\text{-}C_4H_9NH_2)_2]$	64	1	Inactive			
	32	1-9	Inactive			

Table 5.3: Continued

Complex	optium dose (mg kg^{-1})	Schedule (days)	Median survival (%T/C)	Therapeutic ratio (MTD-MED)	Toxicity	
					(BUN)	(WBC)
cis[$PtCl_2(C_4H_7NH_3)_2$]	32	1	157	4	+	±
	16	1-9	221	4		
[Pt(mal) (1,2-DAC)]	32	1	154	-		
	16	1-9	254	4		
[Pt (mal) ($C_5H_9NH_2)_2$]	128	1	-	-		
	128	1-9	-	-		
[Pt (OHmal) ($NH_3)_2$]	64	1	150	1	±	+
	32	1-9	200	2	+	+
[Pt (OHmal) (i-$C_5H_{11}NH_2)_2$]	128	1	-	-		
	128	1-9	-	-		
[Pt (Etmal) ($NH_3)_2$]	128	1	171	2	+	±
	64	1-9	186	4	+	+
[Pt (1,1-CBDCA) ($NH_3)_2$]	128	1	150	2	+	±
	64	1-9	157	2	+	+

[a]+ Denotes toxicity lower than for cis Pt(II).

[b]± Denotes toxicity comparable with cis Pt(II).

[c]trans (OH) groups.

Table 5.4 :

Screening Data for Platinum Complexes Against L1210 Tumor (10^6 Cells) in BDF_1 Mice

Complex	optium dose ($mg\ kg^{-1}$)	Schedule (days)	Median survival (%T/C)	Therapeutic ratio (MTD-MED)
[Pt(mal) (1,2-DAC)][a]	32	1	154	-
	16	1-9	254	4
$[Pt(ClAc)_2\ (i\text{-}C_3H_7NH_2)_2]$[a]	32	1	179	4
	16	1-9	207	8
$[Pt(ClAc)_2\ (C_6H_9NH_2)_2]$[a]	128	1	-	-
	32	1-9	143	1
$[Pt(NO_3)_2\ (i\text{-}C_3H_7NH_2)_2]$[a]	64	1	171	4
	16	1-9	193	4
$[Pt(SO_4)\ (H_2O)\ (1,2\text{-DAC})]$[b]	4	1	119	-
	5	1	116[c]	-
	3.33	1,5,9	285[c]	-
	0.6	1-9	239[c]	-

[a]Testing data of Bristol Labortaries.

[b]Data of Gale et all., U.S. Patent Appl. 769,888.

[c]10^5 cells injected.

Table 5.5 :

Multiple Tumor Screening Data for $[PtCl_2(OH)_2(i\text{-}C_3H_7\&NH_2)_2]$ of Reported Antitumor Studies

Tumer	Host	Parameter	Schedule (days)	Optium dose ($mg\text{-}kg^{-1}$)	%T/C
B16 melanoma	BDF_1	MDST	1-9	12.5	164
P388	CDF_1 (a)	MDST	1-9	18.0	202
	CDF_1 (a)	MDST	1,5,9	50	154
L1210	BDF_1 (a)	MDST	1	32	171
	CDF_1 (b)	MST	1	50	137
	BDF_1 (b)	MST	1-5	20	149
	BDF_1 (a)	MDST	1-9	16	207
	CDF_1 (b)	MST	1-9	25	191
Lewis lung carcinoma	BDF_1 (b)	MDST	1-9	6.25	127
Colon 26	CDF_1	MDST	1,5,9	25	161
Colon 38	BDF_1	MTW	1,8,15	25	52
Mammary	CDF_1	MTW	1,8,15,etc.	50	8
L1210/cytoxan (c)	BDF_1 (b)	MST	1-5	30	166
L1210/BCNU(c)	BDF_1 (b)	MST	1-5	20	139
L1210/L-sarcolysine	BDF_1 (b)	MST	1-5	20	118

(a) 10^6 cells.

(b) 10^5 cells.

Resistant to drug shown.

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